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By:

Christine Manchester

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NEW PATENT APPLICATION TRANSMITTAL

- 1. Transmitted herewith for filing is a:
 - (a) [X] utility patent application converted from U.S. Provisional Application Serial Nos. 60/079,965, filed March 30, 1998 and 60/113,146, filed December 16, 1998.
 - [] design patent application
 - [] plant patent application
 - (b) Inventor(s): John D. Baxter; Beatrice Darimont; Weijun Feng; Robert J. Fletterick; Peter J. Kushner; Richard L. Wagner; Brian L. West; and Keith R. Yamamoto

For: METHODS AND COMPOUNDS FOR MODULATING NUCLEAR RECEPTOR COACTIVATOR BINDING

(c) [X] 19 sheets of formal drawing(s).

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METHODS AND COMPOUNDS FOR MODULATING NUCLEAR RECEPTOR COACTIVATOR BINDING

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INTRODUCTION

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Technical Field

The present invention relates to methods and compounds for modulating nuclear receptor coactivator binding.

Background

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Cells contain receptors that can elicit a biological response by binding various molecules including proteins, hormones and/or drugs. Nuclear receptors represent a super family of proteins that are hormone/ligand-activated transcription factors that enhance or repress transcription in a cell type-, ligand- and promoter-dependent manner. The nuclear receptor family includes receptors for glucocorticoids (GRs), androgens (ARs), mineralocorticoids (MRs), progestins (PRs), estrogens (ERs), thyroid hormones (TRs), vitamin D (VDRs), retinoids (RARs and RXRs), peroxisomes (XPARs and PPARs) and icosanoids (IRs). The so-called "orphan receptors" for which ligands have not been identified are also part of the nuclear receptor superfamily, as they are structurally homologous to the classic nuclear receptors, such as steroid and thyroid receptors.

Although overall sequence conservation between nuclear receptors varies between different families of receptors, sequence conservation between functional regions, or modules, of the receptors is high. For example, nuclear receptors can be organized into functional modules comprising an N-terminal transcriptional activation domain, a central DNA binding domain (DBD), and a C-terminal ligand binding domain (LBD). The LBD of nuclear receptors represents a hormone/ligand-dependent molecular switch. Binding of hormone to a nuclear receptor's LBD changes its ability to modulate transcription of DNA, although they may have transcription-independent actions. Nuclear receptors also bind proteins, such as chaperone complexes, corepressors, or coactivators, that are involved in receptor function. Hormone binding by a nuclear receptor can increase or decrease binding affinity to these proteins, and can influence or mediate the multiple actions of the nuclear receptors on transcription. For example, nuclear receptors can stimulate transcription in response to hormone binding by recruiting coactivator proteins to promoters of responsive genes (Glass et al., *Curr. Opin. Cell Biol.* (1997) 9:222-32); and Horwitz et al., *Mol. Endocrinol.* (1996) 10:1167-77).

Coactivators of the p160 family mediate activity of a transcriptional activation domain, called AF2, that is part of the nuclear receptor's LBD. A few receptor mutants deficient in coactivator-dependent activation have been isolated (TR: Collingwood et al. *Proc. Natl. Acad. Sci.* (1997) 94:248-253; VDR: Jurutka et al., J. *Biol. Chem.* (1997) 227:14592-14599, Masayama et al., *Mol. Endocrinol.* (1997) 11:1507-1517; ER and RAR: Henttu et al., *Mol. Cell Biol.* (1997) 17:1832-1839). While these studies support the physiological relevance of the observed interaction, the structural and functional nature of the site to which coactivators bind has not been defined.

The medical importance of nuclear receptors is significant. They have been implicated in breast cancer, prostate cancer, cardiac arrhythmia, infertility, osteoporosis, hyperthyroidism, hypercholesterolemia, obesity and other conditions. However, limited treatments are available and current agonist/antagonist drugs used to target nuclear receptors are ligands that bind to the receptor's LBD buried deep within the receptor. Although additional targets on nuclear receptors are desired for drug development, the structural and functional basis of such sites, including the coactivator binding site, has not been described.

Accordingly, a need exists for identification and characterization of the coactivator binding sites of nuclear receptors, and molecules that affect their interaction with cellular coactivator proteins. This would provide a major new target for iterative drug design, synthesis, and selection. It also would be advantageous to devise methods and compositions for reducing the time required to discover compounds that target the coactivator binding site of nuclear receptors and administer them to organisms to modulate physiological processes regulated by nuclear receptors.

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5 Relevant Literature

Wagner et al., (*Nature* (1995) 378:690-697) disclose the crystal structure of rat TR-alpha LBD. Various references disclose mutations in carboxyl-terminal helices of nuclear receptors (Henttu et al., *supra*; O'Donnell et al., *Mol. Endocrinol.* (1991) 5:94-99; Whitfield et al., *Mol. Endocrinol.* (1995) 9:1166-79; Saatcioglu et al., *Mol. Cell Biol.* (1997) 17:4687-95; Collingwood et al., *supra*; Kamei et al., *Cell* (1996) 85:403-14). Hong et al. (*Proc. Natl. Acad. Sci. USA* (1996) 93(10):498-49452) and Hong et al. (*Mol. Cell. Biol.* (1997) 17:2735-2744) disclose cloning and expression of GRIP1 coactivator. Torchia et al., (*Nature* (1997) 387:677-84), Le Douarin et al., (*EMBO J* (1996) 15:6701-6715) and Heery et al. (*Nature* (1997) 387:733-736) disclose sequence alignment of various coactivator proteins showing a (SEQ ID NO: 1) LxxLL motif.

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SUMMARY OF THE INVENTION

The present invention relates to identification and manipulation of the coactivator binding site of nuclear receptors. Identification of this site permits design and obtention of compounds that bind to the coactivator binding site of nuclear receptors and modulate coactivator binding to the receptor. The compounds include agonists and antagonists that modulate nuclear receptor activity by promoting (agonists) or blocking (antagonists) hormone-dependent coactivator binding to the receptor, particularly antagonists. The compounds of the invention can be receptor-, cell- and/or tissue-specific.

The present invention also includes protein cocrystals of nuclear receptors with a molecule bound to the coactivator binding site and methods for making them. The cocrystals provide means to obtain atomic modeling information of the specific amino acids and their atoms forming the coactivator binding site and that interact with molecules that bind to the site, such as coactivator. The cocrystals also provide modeling information regarding the coactivator:nuclear receptor interaction, as well as the structure of coactivators bound thereto.

The present invention further provides methods for identifying and designing small molecules that bind to the coactivator binding site using atomic models of nuclear receptors. The method involves modeling test compounds that fit spacially into a nuclear receptor coactivator binding site of interest using an atomic structural model comprising a nuclear receptor coactivator binding site or portion thereof, screening the test compounds in a biological assay characterized by binding of a test compound to a nuclear receptor coactivator binding site, and identifying a test compound that modulates coactivator binding to the nuclear receptor.

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The invention also includes compositions and methods for identifying coactivator binding sites of nuclear receptors. The methods involve examining the surface of a nuclear receptor of interest to identify residues that modulate coactivator binding. The residues can be identified by homology to the coactivator binding site of human TR described herein. Overlays and superpositioning with a three dimensional model of a nuclear receptor LBD, or a portion thereof that contains a coactivator binding site, also can be used for this purpose. Additionally, alignment and/or modeling can be used as a guide for the placement of mutations on the LBD surface to characterize the nature of the site in the context of a cell.

Also provided is a method of modulating the activity of a nuclear receptor. The method can be *in vitro* or *in vivo*. The method comprises administering, *in vitro* or *in vivo*, a sufficient amount of a compound that binds to the coactivator binding site. Preferred compounds bind to the site with greater affinity than coactivator proteins found in a cell of interest. Binding at this site, the compound can compete for binding of coactivator proteins, thereby inhibiting gene transcription, or in some cases promoting it, even when hormone is or is not bound.

The invention further includes a method for identifying an agonist or antagonist of coactivator binding to a nuclear receptor. The method comprises providing the atomic coordinates comprising a nuclear receptor coactivator binding site or portion thereof to a computerized modeling system; modeling compounds which fit spacially into the nuclear receptor coactivator binding site; and identifying in an assay for nuclear receptor activity a compound that increases or decreases activity of the nuclear receptor through binding the coactivator binding site.

Also provided is a machine-readable data storage medium with information for constructing and manipulating an atomic model comprising a coactivator binding site or portion thereof. The medium comprises a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using said data, is capable of displaying a graphical three-dimensional representation of a molecule or molecular complex for a nuclear receptor coactivator binding site.

Also provided is a method of identifying a compound that selectively modulates the activity of one type of nuclear receptor compared to other nuclear receptors. The method is exemplified by modeling test compounds that fit spacially and preferentially into a nuclear receptor coactivator binding site of interest using an atomic structural model of a nuclear receptor coactivator binding site, selecting a compound that interacts with one or more residues of the coactivator binding site unique in the context of that site, and identifying in an assay for coactivator binding activity a compound that selectively binds to the coactivator binding site compared to other nuclear receptors.

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5 The unique features involved in receptor-selective coactivator binding can be identified by comparing atomic models of different receptors or isoforms of the same type of receptor.

The invention finds use in the selection and characterization of peptide, peptidomimetic, as well as other small molecule compounds, such as small organic molecules, identified by the methods of the invention, particularly new lead compounds useful in treating nuclear receptor-based disorders.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the specific effects of mutations on hTRβ1 transcriptional activation in HeLa cells and correlation with effects on binding to GST-GRIP1. T₃ dependent activation of transcription of a reporter gene, expressed as the percentage of WT is plotted for each mutant. GST-GRIP1 binding, analyzed by autoradiography after separation using 10% SDS-PAGE, was also expressed as the percentage of WT and plotted for each mutant. The GST-GRIP1 used included GRIP1 amino acids 721-1121; the same results were obtained using a GST-GRIP1 construct including GRIP1 amino acids 563-1121 (data not shown).

Figure 2 shows that overexpression of full-length GRIP1 rescues loss of transcriptional activation by hTR β 1 mutants. Indicated amounts of the expression vector for full-length GRIP1, pSG5-GRIP1, is included in the cotransfections, which otherwise are performed as in Figure 1. The WT or different representative hTR β 1 mutants are indicated.

Figure 3 shows specific hERα surface mutants cause loss of transcriptional activation in

HeLa cells in parallel with their loss of *in vitro* GRIP1 binding. The fold E₂ activation, expressed as the percentage of WT, and the phosphorimager quantitation of *in vitro* binding of [³⁵S]-labeled hERα WT and mutants to GST-GRIP1 (GRIP1 amino acids 721-1121) also expressed as the percentage of WT is plotted for each mutant.

Figure 4 shows a plot of the fold E₂ activation observed when the indicated amounts of the full-length GRIP1 expression vector, pSG5-GRIP1, are added to the co-transfection experiment, which otherwise is performed as for Figure 3. The WT or different hERα mutants are indicated.

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The data represent the averages of three independent experiments, with standard deviations less than 10%.

Figure 5 shows a CPK model of the $TR\alpha$ -LBD, indicating the LBD surface locations of mutations made in the full-length hTRβ1. Mutated residues having no effect on GRIP1 binding or effect on activation in HeLa cells are shaded gray. Mutated residues with diminished GRIP1 and SRC-1a binding and diminished activation in HeLa cells are colored to reflect chemical properties of the residues: red, blue (purple), and green indicate acidic, basic, and hydrophobic residues, respectively. The main chain structures of the $TR\alpha$ - and $TR\beta$ -LBDs are the same (data not shown).

Figure 6 shows sequence alignment of amino acid residues of members of the p160 coactivator family. Single amino acid designations are used. Members of the p160 coactivator family interact with the nuclear receptors through conserved (SEQ ID NO: 1) LxxLL motifs.

Figure 7 shows binding affinity assays of GST-GRIP1 constructs with NR-boxes 1, 2, and/or 3 and their interaction with TR LBD. GRIP-1 NR boxes 1,2 and 3 interact differently with TR β LBD. Single letter designations are used for the amino acids.

Figure 8 shows binding affinity assays of GST-GRIP1 constructs with NR-boxes 1, 2, and/or 3 and their interaction with TR and GR LBDs. TR and GR differ in their interactions with GRIP-1.

Figure 9 shows binding affinity assays for NR-box 2- and 3-peptides and GRIP1 and their interaction with TR LBD. NR box 2- and 3-containing peptides reproduce the affinity and specificity of the NR interaction domain.

Figure 10 shows binding affinity assays for NR-box 2- and 3-peptides and their interaction with TR LBD. Sequence adjacent to the (SEQ ID NO: 1) LxxLL motif modulate the affinity of NR-box-TRβ LBD interactions.

Figure 11 shows binding affinity assays for mutant GRIP1 and NR-box 2- and 3-peptides and their interaction with TR LBD. The individual leucine residues of the (SEQ ID NO: 1) LxxLL motif are crucial for binding of the GRIP-1 NR interaction domain to TRβ LBD.

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Figure 12 shows the contents of the asymmetric unit of the crystallized hTRß LBD:GRIP1 NR-box 2 peptide complex. The crystal lattice consists of a repeating unit containing a 2:2 complex of hTR LBD and GRIP1 site 2 peptide. Positions of the two GRIP1 site 2 peptides are boxed, in green (site1), and red (site 2), with the peptides drawn as a C-alpha trace. The two NCS related monomers of the hTR LBD are shown as a secondary structure ribbon drawing, with monomer 1 in light grey, and monomer 2 in dark grey. The side chains of the hydrophobic residues I689, L690, L693, L694 of the GRIP1 NR-box 2 peptides are drawn to emphasize those interactions observed in both bound peptides.

Figure 13 shows a ribbon diagram depicting the interaction of the GRIP1 NR-box 2 peptide with the hTRß LBD. The GRIP1 NR-box 2 peptide (dark grey) forms three turns of α-helix, and binds the hTR LBD (light gray) in a hydrophobic cleft defined by helices H3, H4, H5, and H12. Portions of the hTRß LBD, and the neighboring monomer, are omitted for clarity.

Figure 14 shows interface between the GRIP1 NR-box 2 peptide and the hTRß LBD. Side chains of residues of the hTRß LBD within 4.5Å of the GRIP-1 NR-box 2 peptide are labeled. The color of the individual side chains reflects the chemical nature of the residue: acidic residues are red, basic residue are blue, aliphatic residues are green, aromatic residues are brown, and polar residues are orange. The peptide is depicted as a C-alpha trace with the side chains of (SEQ ID NO: 2) ILxxLL motif shown explicitly.

Figure 15 shows residues in the hTRß LBD that are necessary for transactivation. The transactivation mutations are mapped onto the interface between the GRIP1 NR-box 2 peptide and the hTRβ LBD.

Figure 16 shows molecular surface of the hTR LBD. The side chains of the leucines resides fit within a hydrophobic groove formed from helices H3, H5, and H12, while the side chain of the non-conserved isoleucine residue packs against the outside edge of the groove. The remainder of the peptide is shown as main chain.

Figure 17 shows complementarity between the (SEQ ID NO: 1) LxxLL motif and the surface of the hTR LBD. The side chains of the (SEQ ID NO: 2) ILxxLL motif are shown in a CPK representation, with the main chain of the peptide drawn as a C-alpha trace. The three leucince

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residues fit into pockets of the coactivator binding site of the hTRß LBD, depicted as mesh, while the isoleucine residue rests on the edge of the site's cleft.

Figure 18 shows the coactivator binding site cleft, one side of which is formed by conformationally hormone-responsive residues. On the left is a view of the TR-LBD molecular surface showing the concave surfaces in gray. The cavity is shown at the center of the figure. On the right is shown a CPK model of the TR-LBD, overlaid with a molecular surface view, which is restricted to a 12Å radius of the hydrophobic cavity. Mutated residues of the coactivator binding site that are hormone-insensitive (V284, K288, I302 and K306) are located on one side of the cleft and are colored yellow. Mutated CBS residues likely undergo a conformational change upon hormone binding (L454 and E457) are located on the opposite side of the cleft and are colored red.

Figure 19 shows alignment of amino acid sequences (single letter amino acid designations) containing residues that form the coactivator binding sites of several nuclear receptors. The boxes represent residues of alpha-helix (H3, H4, H5, H6 and H12); lower case letters "h" and "q" represent hydrophobic and polar residues, respectively.

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DESCRIPTION OF SPECIFIC EMBODIMENTS

The present invention provides methods and compositions for identifying compounds that modulate nuclear receptor activity. The compounds can be nuclear receptor agonists or antagonists that bind to the coactivator binding site (and that act as mimetics to the coactivator in this regard), and promote (agonists) or block (antagonists) binding of the coactivator to the target nuclear receptor. Compounds that bind to the coactivator binding site also are provided. The compounds can be natural or synthetic. Preferred compounds are small organic molecules, peptides and peptidomimetics (e.g., cyclic peptides, peptide analogs, or constrained peptides).

As described in the Examples, mutagenesis and coactivator binding studies, coupled with analysis of atomic models derived from cocrystals, reveals for the first time a previously unknown structure for nuclear receptors, the coactivator binding site. By "coactivator binding site" is intended a structural segment or segments of nuclear receptor polypeptide chain folded in such a way so as to give the proper geometry and amino acid residue conformation for binding a coactivator. This is the physical arrangement of protein atoms in three-dimensional space forming a coactivator binding site pocket or cavity. Residues forming the site are amino acids corresponding to (i.e., the same as or equivalent to) human TR residues of C-terminal helix 3 (Ile280, Thr281, Val283, Val284, Ala287, and Lys288), helix 4 (Phe293), helix 5 (Gln301, Ile302, Leu305, Lys306), helix 6 (Cys309), and helix 12 (Leu454, Glu457, Val458 and Phe459). The coactivator binding site is highly conserved among the nuclear receptor super family (Figure 19). Thus, this site corresponds to a surprisingly small cluster of residues on the surface of the LBD that form a The hydrophobic cleft is formed by hydrophobic residues prominent hydrophobic cleft. corresponding to human TR residues of C-terminal helix 3 (Ile280, Val283, Val284, and Ala287), helix 4 (Phe293), helix 5 (Ile302 and Leu305), helix 6 (Cys309), and helix 12 (Leu454, Val458 and Phe459). The hydrophobic cleft of the coactivator binding site also is highly conserved among the nuclear receptor super family (Figure 19).

The invention also includes compositions and methods for identifying coactivator binding sites of nuclear receptors. The methods involve examining the surface of a nuclear receptor of interest to identify residues that modulate coactivator binding. The residues can be identified by homology to the coactivator binding site of human TR described herein. A preferred method is alignment with the residues of any nuclear receptor corresponding to (i.e., equivalent to) human TR residues of the C-terminal helix 3 (Ile280, Thr281, Val283, Val284, Ala287, and Lys288), helix 4

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(Phe293), helix 5 (Gln301, Ile302, Leu305, Lys306), helix 6 (Cys309), and helix 12 (Pro453, Leu454, Glu457, Val458 and Phe459). Overlays and superpositioning with a three-dimensional model of a nuclear receptor LBD, or a portion thereof that contains a coactivator binding site, also can be used for this purpose. For example, three-dimensional structures of TR, RAR, RXR and ER LBDs can be used for this purpose. For example, nuclear receptors identifiable by homology alignment include normal nuclear receptors or proteins structurally related to nuclear receptors found in humans, natural mutants of nuclear receptors found in humans, normal or mutant receptors found in animals, as well as non-mammalian organisms such as pests or infectious organisms, or viruses.

Alignment and/or modeling also can be used as a guide for the placement of mutations on the LBD surface to characterize the nature of the site in the context of a cell. Selected residues are mutated to preserve global receptor structure and solubility. To destroy the coactivator binding interaction, preferred mutations are to charged residues (e.g., Arg, Lys, or Glu) on the basis that bulky, surface charged residues might disrupt coactivator binding, yet preserve global receptor structure and solubility. Mutants can be tested for coactivator binding as well as the relative change in strength of the binding interaction. Ligand-dependent coactivator interaction assays also can be tested for this purpose, such as those described herein.

Compounds that bind to the coactivator binding site of nuclear receptors can be identified by computational modeling and/or screening. For example, coactivator agonists or antagonists can be identified by providing atomic coordinates comprising a nuclear receptor coactivator binding site or portion thereof to a computerized modeling system, modeling them, and identifying compounds that fit spacially into the coactivator binding site. By a "portion thereof" is intended the atomic coordinates corresponding to a sufficient number of residues or their atoms of the coactivator binding site that interact with a compound capable of binding to the site. This includes receptor residues having an atom within 4.5Å of a bound compound or fragment thereof. For instance, human TR residues V284, Phe293, Ile302, Leu305 and Leu454 contain side chain atoms that are within 4.5Å, and interact with, hydrophobic residues of a (SEQ ID NO: 1) LxxLL motif of an NRbox 2 coactivator peptide. As another example, an atomic structural model utilized for computational modeling and/or screening of compounds that bind to the coactivator binding site may include a portion of atomic coordinates of amino acid residues corresponding to the site composed of residues of human thyroid receptor selected from Val284, Lys288, Ile302, Lys306, Leu454 and Glu457, or their structural and functional equivalents found in other receptors. Thus, for example, the atomic coordinates provided to the modeling system can contain atoms of the nuclear receptor LBD, part of the LBD such as atoms corresponding to the coactivator binding site

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or a subset of atoms useful in the modeling and design of compounds that bind to a coactivator binding site.

The atomic coordinates of a compound that fits into the coactivator binding site also can be used for modeling to identify compounds or fragments that bind the site. By "modeling" is intended quantitative and qualitative analysis of molecular structure/function based on atomic structural information and receptor-coactivator agonists/antagonists interaction models. This includes conventional numeric-based molecular dynamic and energy minimization models, interactive computer graphic models, modified molecular mechanics models, distance geometry and other structure-based constraint models. Modeling is preferably performed using a computer and may be further optimized using known methods. By "fits spacially" is intended that the three-dimensional structure of a compound is accommodated geometrically by a cavity or pocket of a nuclear receptor coactivator binding site.

Compounds of particular interest fit spacially and preferentially into the coactivator binding By "fits spacially and preferentially" is intended that a compound possesses a threedimensional structure and conformation for selectively interacting with a nuclear receptor coactivator binding site. Compounds that fit spacially and preferentially into the coactivator binding site interact with amino acid residues forming the hydrophobic cleft of this site. In particular, the hydrophobic cleft of the coactivator binding site comprises a small cluster of hydrophobic residues. The site also contains polar or charged residues at its periphery. The present invention also includes a method for identifying a compound capable of selectively modulating coactivator binding to different nuclear receptors. The method comprises the steps of modeling test compounds that fit spacially and preferentially into the coactivator binding site of a nuclear receptor of interest using an atomic structural model of a nuclear receptor, screening the test compounds in a biological assay for nuclear receptor activity characterized by preferential binding of a test compound to the coactivator binding site of a nuclear receptor, and identifying a test compound that selectively modulates the activity of a nuclear receptor. Such receptor-specific compounds are selected that exploit differences between the coactivator binding sites of one type of receptor versus a second type of receptor, such as the differences depicted in Figure 19.

The invention also is applicable to generating new compounds that distinguish nuclear receptor isoforms. This can facilitate generation of either tissue-specific or function-specific compounds. For instance, GR subfamily members have usually one receptor encoded by a single gene, although there are exceptions. For example, there are two PR isoforms, A and B, translated from the same mRNA by alternate initiation from different AUG codons. There are two GR forms,

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one of which does not bind ligand. This method is especially applicable to the TR subfamily which usually has several receptors that are encoded by at least two (TR: α , β) or three (RAR, RXR, and PPAR: α , β , γ) genes or have alternate RNA splicing.

The receptor-specific compounds of the invention preferably interact with conformationally constrained residues of the coactivator binding site that are conserved among one type of receptor compared to a second type of receptor. "Conformationally constrained" is intended to refer to the three-dimensional structure of a chemical or moiety thereof having certain rotations about its bonds fixed by various local geometric and physical-chemical constraints. Conformationally constrained structural features of a coactivator binding site include residues that have their natural flexible conformations fixed by various geometric and physical-chemical constraints, such as local backbone, local side chain, and topological constraints. These types of constraints are exploited to restrict positioning of atoms involved in receptor-coactivator recognition and binding.

For instance, comparison of sequences of the GR and TR coactivator interaction surface shows a highly negatively charged sequence at the C-terminal end of TR helix 12 (E460 and D461) that is neutral in the equivalent positions in GR helix 12 (GR residues T788 and N759, corresponding to TR residue positions 460 and 461, as depicted in Figure 19). As described in the Examples, the cocrystal of the hTRß LBD complexed with the GRIP1 NR-box 2 peptide shows that TR residues E460 and D461 interact with positively charged residues of the NR-box 2 peptide. Also, when comparing the RAR LBD structure to that of the TR LBD, conformation of helix 12 differs slightly, whereas helices 3, 4, 5 and 6 are substantially the same. Thus, differences in helix 12, particularly charge differences at the C-terminal end of the helix, may modulate preferential interaction of TR for NR-box 2 containing coactivators. As further demonstrated in the Examples, TR and GR differ in their specificity for different NR-boxes containing the conserved (SEQ ID NO: 1) LxxLL motif found in members of the p160 family of coactivator proteins. As also demonstrated in the Examples, GR but not TR is able to interact with peptides containing the hydrophobic interaction motifs of p53 (SEQ ID NO: 3; FxxLW) and VP16 (SEQ ID NO: 4; FxxAL). Thus, TR exhibits preferential interaction with NR-box peptides comprising the (SEQ ID NO: 1) LxxLL motif, but GR does not discriminate and can bind peptides containing a generic amphipathic helix motif. Accordingly, these real differences among the various nuclear receptors can be exploited in the identification and design of compounds that modulate coactivator binding to one nuclear receptor compared to another.

For modeling, docking algorithms and computer programs that employ them can be used to identify compounds that fit into the coactivator binding site. For example, docking programs can be

used to predict how a small molecule of interest can interact with the nuclear receptor coactivator binding site. Fragment-based docking also can be used in building molecules *de novo* inside the coactivator binding site, by placing chemical fragments that complement the site to optimize intermolecular interactions. The techniques can be used to optimize the geometry of the binding interactions. This design approach has been made possible by identification of the coactivator binding site structure thus, the principles of molecular recognition can now be used to design a compound which is complementary to the structure of this site. Compounds fitting the coactivator binding site serve as a starting point for an iterative design, synthesis and test cycle in which new compounds are selected and optimized for desired properties including affinity, efficacy, and selectivity. For example, the compounds can be subjected to addition modification, such as replacement and/or addition of R-group substituents of a core structure identified for a particular class of binding compounds, modeling and/or activity screening if desired, and then subjected to additional rounds of testing.

Computationally small molecule databases can be screened for chemical entities or compounds that can bind in whole, or in part, to a nuclear receptor coactivator binding site of interest. In this screening, the quality of fit of such entities or compounds to the binding site may be judged either by shape complementarity (DesJalais et al., *J. Med. Chem.* (1988) 31:722-729) or by estimated interaction energy (Meng et al., *J. Comp. Chem.* (1992) 13:505-524). The molecule databases include any virtual or physical database, such as electronic and physical compound library databases, and are preferably used in developing compounds that modulate coactivator binding.

Compounds can be designed intelligently by exploiting available structural and functional information by gaining an understanding of the quantitative structure-activity relationship (QSAR), using that understanding to design new compound libraries, particularly focused libraries having chemical diversity of one or more particular groups of a core structure, and incorporating any structural data into that iterative design process. For example, one skilled in the art may use one of several methods to screen chemical entities or fragments for their ability to associate with the coactivator binding site of a nuclear receptor of interest. This process may begin by visual inspection of, for example, the coactivator binding site on the computer screen. Selected fragments or chemical entities may then be positioned into all or part of the site. Docking may be accomplished using software such as Quanta and Sybyl, followed by energy minimization and molecular dynamics with standard molecular mechanics force-fields, such as CHARMM and AMBER.

For example, compounds and/or fragments can be designed to fill up the hydrophobic cleft, the pocket deep within the cleft, the upper end of the site, and/or the lower end of the site. Residues comprising a coactivator binding site, when defined by the user as those residues having an atom within 4.5Å of an atom of a bound chemical entity, can be modeled to look for energetic contributions and interaction with the bound chemical entity. For example, a compound or fragment can be designed to contain hydrophobic groups that interact with hydrophobic residues of the coactivator binding site. As described in the examples, human TR residues V284, Phe293, Ile302, Leu305 and Leu454 contain side chain atoms that are within 4.5Å, and interact with, hydrophobic residues of a (SEQ ID NO: 1) LxxLL motif of an NR-box 2 coactivator peptide. Thus, for example, peptides and/or peptide mimetics having a hxxhh motif, where "h" is a hydrophobic residue and x is any residue, can be constructed. Small organic molecules that mimic one or more of these particular interactions also can be designed, for example, by including one or more R-groups that are hydrophobic and fit into the site.

Specialized computer programs may also assist in the process of selecting chemical entity fragments or whole compounds. These include: GRID (Goodford, *J. Med. Chem.* (1985) 28:849-857; available from Oxford University, Oxford, UK); MCSS (Miranker et al., *Proteins: Structure, Function and Genetics*, (1991) 11:29-34; available from Molecular Simulations, Burlington, MA); AUTODOCK (Goodsell et al., *Proteins: Structure, Function and Genetics* (1990) 8:195-202; available from Scripps Research Institute, La Jolla, CA); and DOCK (Kuntz et al, *J. Mol. Biol.* (1982) 161:269-288; available from University of California, San Francisco, CA).

Additional commercially available computer databases for small molecular compounds include Cambridge Structural Database and Fine Chemical Database (Rusinko, *Chem. Des. Auto. News* (1993) 8:44-47).

Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound. Assembly may be proceeded by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure coordinates of a nuclear receptor. This can be followed by manual model building using software such as Quanta or Sybyl.

Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include: CAVEAT (Bartlett et al., "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", In: *Molecular Recognition in Chemical and Biological Problems*", Special Pub., *Royal Chem. Soc.* (1989) 78:182-196; CAVEAT is available from the University of California, Berkeley, CA); 3D Database systems such as MACCS-3D (MDL

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Information Systems, San Leandro, CA; reviewed in Martin, *J. Med. Chem.* (1992) *35*:2145-2154); and HOOK (available from Molecular Simulations, Burlington, MA).

In addition to building a compound in a step-wise fashion, one fragment or chemical entity at a time as described above, compounds that bind to a coactivator binding site of interest also may be designed as a whole or *de novo* using either an empty coactivator binding site or optionally including some portion(s) of a molecule known to binds to the site, such as an NR-box type peptide. These methods include: LUDI (Bohm, *J. Comp. Aid. Molec. Design* (1992) 6:61-78; LUDI is available from Biosym Technologies, San Diego, CA); LEGEND (Nishibata et al., *Tetrahedron* (1991) 47:8985; LEGEND is available from Molecular Simulations, Burlington, MA); and LeapFrog (available from Tripos Associates, St. Louis, MO).

Other molecular modeling techniques may also be employed in accordance with this invention. See, for example, Cohen et al., *J. Med. Chem.* (1990) 33:883-894); Navia et al., *Curr. Opin. Struct. Biol.* (1992) 2:202-210). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the structure of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, for example, Farmer, "*Drug Design*," Ariens, E.J., ed., 10:119-143 (Academic Press, New York, 1980); U.S. Patent No. 5,331,573; U.S. Patent No. 5,500,807; Verlinde, *Structure*, (1994) 2:577-587); and Kuntz et al., *Science*, (1992) 257:1078-1082). The model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Using these computer modeling systems a large number of compounds may be quickly and easily examined and expensive and lengthy biochemical testing avoided. Moreover, the need for actual synthesis of many compounds can be substantially reduced and/or effectively eliminated.

Compounds identified through modeling can be screened in an assay characterized by binding of the compound to a coactivator binding site of interest for coactivator binding activity, such as a biologically based assay. Screening can be *in vitro* and/or *in vivo*. Preferred assays include cell-free competition assays and cell culture based assays. The biological screening preferably centers on activity-based response models, binding assays (which measure how well a compound binds to the receptor), and bacterial, yeast and animal cell lines (which measure the biological effect of a compound in a cell). The assays can be automated for high capacity - high throughput screening (HTS) in which large numbers of compounds can be tested to identify compounds with the desired activity.

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As an example, in vitro binding assays can be performed in which compounds are tested for their ability to block the binding of a coactivator protein, fragment, fusion or peptide thereof, to a coactivator binding site of interest. For cell and tissue culture assays, they may be performed to assess a compound's ability to block function of cellular coactivators, such as members of the p160 family of coactivator proteins, such as SRC-1, AIB1, RAC3, p/CIP, and GRIP1 and its homologues TIF 2 and NcoA-2, and those that exhibit receptor and/or isoform-specific binding affinity. In a preferred embodiment, compounds of the invention bind to a nuclear receptor coactivator binding site with greater affinity than the cellular coactivator proteins. Tissue profiling and appropriate animal models also can be used to select compounds. Different cell types and tissues also can be used for these biological screening assays. Suitable assays for such screening are described herein and in Shibata et al. (Recent Prog. Horm. Res. 52:141-164 (1997)); Tagami et al. (Mol. Cell Biol. (1997) 17(5):2642-2648); Zhu et al. (J. Biol. Chem. (1997) 272(14):9048-9054); Lin et al. (Mol. Cell Biol. (1997) 17(10):6131-6138); Kakizawa et al. (J. Biol. Chem. (1997) 272(38):23799-23804); and Chang et al. (Proc. Natl. Acad. Sci. USA (1997) 94(17):9040-9045), which references are incorporated herein in their entirety by reference. For example, coactivators or binding fragments thereof can be expressed and/or assayed for binding as for GRIP1 (Hong et al., MCB supra; and Hong et al., PNAS supra) and/or SRC-1 (Spencer et al., Nature (1997) 389:194-198; Onate et al., Science (1995) 270:1354-1357), incorporated by reference.

The compounds selected can have agonist and/or antagonistic properties. The compounds also include those that exhibit new properties with varying mixtures of agonist and antagonist activities, depending on the effects of altering coactivator binding in the context of different activities of nuclear receptors, either hormone-dependent or hormone-independent, which are mediated by proteins other than coactivators, and which interact with the receptors at locations other than the coactivator binding site. The compounds also include those, which through their binding to receptor locations that are conformationally sensitive to hormone binding, have allosteric effects on the receptor by stabilizing or destabilizing the hormone-bound conformation of the receptor, or by directly inducing the same, similar, or different conformational changes induced in the receptor by the binding of hormone.

Of particular interest is use of such compounds in a method of modulating nuclear receptor activity in a mammal by administering to a mammal in need thereof a sufficient amount of a compound that fits spatially and preferentially into a coactivator binding site of a nuclear receptor of interest. By "modulating" is intended increasing or decreasing activity of a nuclear receptor. For example, pre-clinical candidate compounds can be tested in appropriate animal models in order to measure efficacy, absorption, pharmacokinetics and toxicity following standard techniques known

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in the art. Compounds exhibiting desired properties are then tested in clinical trials for use in treatment of various nuclear receptor-based disorders. These include ER-based disorders, such as postmenopausal symptoms and cancer resulting from loss of estrogen production, and osteoporosis and cardiovascular disease stemming from traditional estrogen replacement therapy. Others include TR-based disorders including cardiovascular disease, metabolic disorders, hyperthyroidism, glaucoma and skin disorders. GR-based disorders include Type II diabetes and inflammatory conditions such as rheumatic diseases.

The invention also provides for cocrystals made from nuclear receptor ligand binding domains with a molecule bound to the coactivator binding site. As exemplified in the Examples, TR LBDs are co-crystallized with a peptide molecule comprising a coactivator NR-box 2 peptide sequence bound to the coactivator binding site, and the hormone/ligand T₃.

Crystals are made from purified nuclear receptor LBDs that are usually expressed by a cell culture, such as *E. coli*. Preferably, different crystals (cocrystals) for the same nuclear receptor are separately made using different coactivators-type molecules, such as protein fragments, fusions or small peptides. The coactivator-type molecules preferably contain NR-box sequences necessary for binding to the coactivator binding site, or derivatives of NR-box sequences. Other molecules can be used in co-crystallization, such as small organics that bind to the coactivator or hormone binding site(s). Heavy atom substitutions can be included in the LBD and/or a co-crystallizing molecule.

After the three dimensional structure of the cocrystal is determined, the structural information can be used in computational methods to design synthetic compounds for the nuclear receptor, and further structure-activity relationships can be determined through routine testing using the assays described herein and known in the art.

Since nuclear receptor LBDs may crystallize in more than one crystal form, the structure coordinates of such receptors or portions thereof, as provided in **Appendix 1**, are particularly useful for solving the structure of those other crystal forms of nuclear receptors. They may also be used to solve the structure of mutants or co-complexes of nuclear receptors having sufficient structural similarity.

One method that may be employed for this purpose is molecular replacement. In this method, the unknown crystal structure, may be determined using the structure coordinates of this invention as provided in **Appendix 1**. This method will provide an accurate structural form for the unknown crystal more quickly and efficiently than attempting to determine such information *ab initio*.

Atomic coordinate information gleaned from the crystals of the invention can be stored. In a preferred embodiment, the information is provided in the form of a machine-readable data storage medium. This medium contains information for constructing and/or manipulating an atomic model of a coactivator binding site or portion thereof. For example, the machine readable data for the coactivator binding site comprises structure coordinates of amino acids corresponding to human TR amino acids selected from C-terminal helix 3 (Ile280, Thr281, Val283, Val284, Ala287, and Lys288), helix 4 (Phe293), helix 5 (Gln301, Ile302, Leu305, Lys306), helix 6 (Cys309), and helix 12 (Pro453, Leu454, Glu457, Val458 and Phe459), or a homologue of the molecule or molecular complex comprising the site. The homologues comprise a coactivator binding site that has a root mean square deviation from the backbone atoms of the amino acids of not more than 1.5Å. A preferred molecule or complex represents a compound bound to the coactivator binding site.

The machine-readable data storage medium can be used for interative drug design and molecular replacement studies. For example, a data storage material is encoded with a first set of machine-readable data that can be combined with a second set of machine-readable data. For molecular replacement, the first set of data can comprise a Fourier transform of at least a portion of the structural coordinates of the nuclear receptor or portion thereof of interest, and the second data set comprises an X-ray diffraction pattern of the molecule or molecular complex of interest. Using a machine programmed with instructions for using the first and second data sets a portion or all of the structure coordinates corresponding to the second data can be determined.

Protein for crystals and assays described herein can be produced using expression and purification techniques described herein and known in the art. For example, high level expression of nuclear receptor LBDs can be obtained in suitable expression hosts such as *E. coli*. Expression of LBDs in *E. coli*, for example, includes the TR LBD and other nuclear receptors, including members of the steroid/thyroid receptor superfamily, such as the receptors ER, AR, MR, PR, RAR, RXR and VDR. Yeast and other eukaryotic expression systems can be used with nuclear receptors that bind heat shock proteins as these nuclear receptors are generally more difficult to express in bacteria, with the exception of ER, which can be expressed in bacteria. Representative nuclear receptors or their ligand binding domains have been cloned and sequenced: human RAR-α, human RAR-γ, human RXR-α, human PPAR-α, human PPAR-β, human PPAR-γ, human VDR, human ER (as described in Seielstad *et al.*, *Molecular Endocrinol.*, (1995) 9:647-658, incorporated herein by reference), human GR, human PR, human MR, and human AR. The LBD for each of these receptors has been identified.

Coactivator proteins can be expressed using techniques known in the art, particularly members of the p160 family of coactivator proteins that have been cloned and/or expressed previously, such as SRC-1, AIB1, RAC3, p/CIP, and GRIP1 and its homologues TIF 2 and NcoA-2. A preferred method for expression of coactivator protein is to express a fragment that retains transcriptional activation activity using the "yeast 2-hybrid" method as described by Hong et al. (PNAS <u>supra</u>; and MCB <u>supra</u>), for GRIP1 expression, which reference is herein incorporated by reference.

The proteins can be expressed alone, as fragments of the mature or full-length sequence, or as fusions to heterologous sequences. For example, TR can be expressed without any portion of the DBD or amino-terminal domain. Portions of the DBD or amino-terminus can be included if further structural information with amino acids adjacent the LBD is desired. Generally, for the TR the LBD used for crystals will be less than 300 amino acids in length. Preferably, the TR LBD will be at least 150 amino acids in length, more preferably at least 200 amino acids in length, and most preferably at least 250 amino acids in length. For example the LBD used for crystallization can comprise amino acids spanning from Met 122 to Val 410 of the rat TR- α or Glu 202 to Asp 461 of the human TR- β .

Typically the LBDs are purified to homogeneity for crystallization. Purity of LBDs can be measured with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), mass spectrometry (MS) and hydrophobic high performance liquid chromatography (HPLC). The purified LBD for crystallization should be at least 97.5 % pure, preferably at least 99.0% pure, more preferably at least 99.5% pure.

Initially, purification of the unliganded receptor can be obtained by conventional techniques, such as hydrophobic interaction chromatography (HPLC), ion exchange chromatography (HPLC), and heparin affinity chromatography.

To achieve higher purification for improved crystals of nuclear receptors, especially the TR subfamily and TR, the receptors can be ligand-shift-purified using a column that separates the receptor according to charge, such as an ion exchange or hydrophobic interaction column, and then bind the eluted receptor with a ligand, especially an agonist. The ligand induces a change in the receptor's surface charge such that when re-chromatographed on the same column, ligand-bound receptor is separated from unliganded receptor. Usually saturating concentrations of ligand are used in the column and the protein can be preincubated with the ligand prior to passing it over the

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column. The structural studies detailed herein indicate the general applicability of this technique for obtaining super-pure nuclear receptor LBDs for crystallization.

Purification can also be accomplished by use of a purification handle or "tag," such as with at least one histidine amino acid engineered to reside on the end of the protein, such as on the N-terminus, and then using a nickel or cobalt chelation column for purification. (Janknecht et al., *Proc. Natl. Acad. Sci. USA*, (1991) 88:8972-8976) incorporated by reference.

Typically purified LBD, such as TR LBD, is equilibrated at a saturating concentration of ligand at a temperature that preserves the integrity of the protein. Ligand equilibration can be established between 2 and 37°C, although the receptor tends to be more stable in the 2-20°C range. Preferably crystals are made with the hanging drop methods detailed herein. Regulated temperature control is desirable to improve crystal stability and quality. Temperatures between 4 and 25°C are generally used and it is often preferable to test crystallization over a range of temperatures. The crystals are then subjected to vapor diffusion and bombarded with x-rays to obtain x-ray diffraction pattern following standard procedures.

For co-crystallization with a peptide that binds to the coactivator binding site, various concentrations of peptides containing a sequence that binds to a coactivator binding site of a nuclear receptor of interest can be used in microcrystallization trials, and the appropriate peptides selected for further crystallization. Any number of techniques, including those assays described herein can assay peptides for binding to the coactivator binding site of a nuclear receptor of interest. In a preferred embodiment, a NR-box 2 sequence-containing peptide is used for crystallization with TR LBD. A preferred peptide contains a NR-box (SEQ ID NO: 1) LxxLL motif, and suitable flanking sequences necessary for binding and forming complex with coactivator binding site of the nuclear receptor of interest, such as a TR LBD. The binding peptides are then tested in crystallization trials at various concentrations and ratios of concentrations with a nuclear receptor of interest, for example, as described herein and in the Examples. For crystallization trials with TR LBD, the hanging drop vapor diffusion method is preferred. Conditions of pH, solvent and solute components and concentrations and temperature can be adjusted, for instance, as described in the Examples. In the handing drop method, to obtain suitable crystals for x-ray diffraction analysis, seeding of prepared drops with microcrystals of the complex can be used. Collection of structural information can be determined by molecular replacement using the structure of TR LBD determined herein or previously by Wagner et al., supra. The structure is refined following standard techniques known in the art.

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There are many uses and advantages provided by the present invention. For example, the methods and compositions described herein are useful for identifying peptides, peptidomimetics or small natural or synthetic organic molecules that modulate nuclear receptor activity. The compounds are useful in treating nuclear receptor-based disorders. Methods and compositions of the invention also find use in characterizing structure/function relationships of natural and synthetic coactivator compounds.

The following examples illustrate various aspects of this invention. These examples do not limit the scope of this invention.

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5 <u>EXAMPLES</u>

Example 1: Expression and purification of wild-type and mutant nuclear receptors and coactivators

A. Human TRβ LBD

Human TRß LBD (His6-E202-D461) was expressed and purified as described (Shiau et al., Gene (1996) 179(2):205-10). Briefly, the protein was expressed from pET (e.g., pET3 and pET28) in BL21DE3 at 14°C, induced at OD(600nm) 0.7 with 1mM IPTG and incubation was extended for 24 hours. Cells were harvested and lysed in 50mM sodium-phosphate buffer (pH 8.0), 0.3M NaCl, 10% glycerol, 25mM ß-merceptoethanol and 0.1mM PMSF as described above. The lysate was cleared by ultracentrifugation (Ti45, 36000 rpm, 1h, 4°C), loaded on a Talon column equilibrated in the sodium phosphate buffer described above, washed with 12mM imidazole and eluted with an imidazole gradient (12 - 300 mM). TRß LBD containing fractions were loaded in 0.6M ammonium sulfate on a TSK-phenyl hydrophobic interaction column and eluted with a reverse ammonium sulfate gradient [0.6 – 0 M] in 50% glycerol and 10% acetonitrile. Fractions containing TRB LBD were tested for hormone binding, pooled and incubated with a 3-fold molar excess of T₃ (Sigma). The hydrophobic interaction run was repeated with liganded receptor under the same conditions. Liganded receptor, which elutes earlier than unliganded receptor, was collected and buffer changed to 20mM Hepes pH7.0, 3mM DTT and $0.1\mu M$ T₃ using NAP columns (Pharmacia). For crystallization, the protein was concentrated by ultrafiltration (Millipore UFV2BGC10 concentrators) to a final concentration of 9mg/ml. The yield was about 9.5mg protein per liter bacterial culture.

B. Human TR mutants

Thirty-seven thyroid receptor mutants were created by synthesizing double-stranded oligonucleotides which encode the mutant sequence and which have ends allowing them to be ligated as a cassette using pairs of the NsiI, PstI, SstI, AlwNI, ApoI, PflMI, BstXI, BseRI, BsmFI, PvuII, NspI, SmaI, PmII, BglII and BsmI restriction sites of the hTRβ1 cDNA sequence, or the 3' plasmid polylinker SalI, or BamHI restriction sites. The hTRβ1 sequences thus mutated were subcloned into the pCMX vector encoding the full-length 461 amino acid hTRβ1 sequence. Some of the mutations of the hTRβ1 in the CMX vector and all three mutations of the hERα in the pSG5-ER-HEGO vector (Tora et al., *EMBO* (1989) 8:1981) were created using Quick Change Site-Directed Mutagenesis Kits (Stratagene). The mutated sequences were verified by DNA sequencing using Sequenase Kits (Stratagene).

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C. Human ERa LBD

The human ERα-LBD 297-554 was overexpressed as described previously (Seielstad, et al., *supra*) in BL21(DE3)pLysS cells transformed with a modified pET-23d-ERG vector that contained the sequence Met-Asp-Pro fused to residues 297 to 554 of the hERα (provided by Paul Sigler of Yale University). Clarified bacterial lysates were adjusted to 3 M in urea and 0.7 M in NaCl and then applied to a 10-ml column of estradiol-Sepharose (Greene, et al., *Proc. Natl. Acad. Sci. USA* (1980) 77:5115-5119; Landel, et al., *Mol. Endocrinol.* (1994) 8:1407-1419; Landel, et al., *J. Steroid Biochem. Molec. Biol.* (1997) 63:59-73).

To carboxymethylate the solvent-accessible cysteines, the bound hERα-LBD was treated with 5 mM iodoacetic acid in 10 mM Tris, pH 8.1, 250 mM NaSCN (Hegy, et al., *Steroids* (1996) 61:367-373). Protein was eluted with 3 x 10-5 M ligand (either DES or OHT) in 30-100 ml of 50 mM Tris, 1 mM EDTA, 1 mM DTT and 250 mM NaSCN, pH 8.5. The yield of hERα-LBD was typically close to 100% (Seielstad, et al., *Biochemistry* (1995) 34:12605-12615). The affinity-purified material was concentrated and exchanged into 20 mM Tris, 1 mM EDTA, 4 mM DTT, pH 8.1 by ultrafiltration. The protein was bound to a Resource Q column (Pharmacia) and then eluted with a linear gradient of 25-350 mM NaCl in 20 mM Tris, pH 8.1, 1 mM DTT. The hERα-LBD-ligand complexes eluted at 150-200 mM NaCl. Pooled fractions were concentrated by ultrafiltration and analyzed by SDS-PAGE, native PAGE, and electrospray ionization mass spectrometry.

D. Human ER mutants

To test the importance of the NR box peptide/LBD interface observed in the crystal, a series of site-directed mutations were introduced into the ERα LBD. These mutations were designed either to simultaneously perturb the structural integrity and the nonpolar character of the floor of the binding groove (Ile 358->Arg, Val 376->Arg and Leu 539->Arg) or to prevent the formation of the capping interactions (Lys 362->Ala and Glu 542->Lys). Fusions of glutathione-S-transferase (GST) to the wild-type and mutant LBDs were analyzed for their ability to bind ³⁵S-labeled GRIP1 in the absence of ligand or in the presence of DES or OHT.

³⁵S-labeled GRIP1 was incubated with either immobilized GST, immobilized wild type GST-hERα LBD, or immobilized mutant GST-LBDs in the absence of ligand or in the presence of DES or OHT. The bound GRIP1 was quantitated after SDS-PAGE. I358R, mutant LBD containing a Ile->Arg substitution at residue 358; K362A, mutant LBD containing a Lys->Ala substitution at residue 362; V376R, mutant LBD containing a Val->Arg substitution at residue 376;

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5 L539R, mutant LBD containing a Leu->Arg substitution at residue 539; E542K, mutant LBD containing a Glu->Lys substitution at residue 542.

In the absence of ligand or in the presence of OHT, fusions to the wild-type protein and all of the mutant LBDs showed no detectable binding to GRIP1. The Ile 358->Arg, Val 376->Arg and Leu 539->Arg mutants were all unable to interact with coactivator in the presence of agonist, confirming the importance of the packing interactions observed in the crystal. Disruption of either the N- or C-terminal capping interaction also compromised GRIP1 binding in the presence of agonist. Only the wild-type GST-LBD was able to recognize the coactivator in the presence of DES.

E. Human ER LBD-GST Fusion Protein

A fusion between glutathione-S-transferase (GST) and amino acids 282-595 of hERα was constructed by subcloning the EcoRI fragment from pSG5 ERα-LBD (Lopez et al., submitted manuscript) into pGEX-3X (Pharmacia). The Ile 358-> Arg, Lys 362->Ala, and Leu 539->Arg mutations were introduced into the GST-LBD construct using the QuikChange Kit (Stratagene) according to the manufacturer's instructions. The Val 376->Arg and Glu 542->Lys mutations were created in the GST-LBD construct by subcloning the BsmI/HindIII fragments of derivatives of pSG5-ER-HEGO (Tora, et al., *supra*) into which these mutations had already been introduced. All constructs were verified by automated sequencing (University of Chicago Cancer Research Center DNA Sequencing Facility).

F. Radiolabeled full-length receptors and coactivator proteins

Wild-type (WT) or mutant pCMV-hTRβ1 vector and the pSG5-GRIP1 and pCMX-SRC-1a vectors were used to produce radiolabeled full-length receptors and coactivator proteins using the TNT coupled Reticulocyte Lysate System (Promega) and [35S]-Met (DuPont). GST-GRIP1 (amino acids 721-1221), GST-GRIP1 (amino acids 563-1121), GST-SRC-1a (amino acids 381-882), GST-hTRβ1 (full-length, WT or mutants, WT provided by. C. Costa), and the GST-hRXRα (full-length provided by. C. Costa), fusion proteins were produced in *E. coli* strain HB101 as per the manufacturer's protocol (Pharmacia Biotech).

G. Coactivator GRIP1 563-767 His6 GST fusion protein

GRIP1 563-767 was cloned as a Bam HI-Xho I fragment derived from pGEX-2TK GRIP1 563-1121 into the corresponding sites of pGEX-4T1. A His6-tag was added by inserting a Xho I-Nae I fragment of pET23a into Xho I-Bsa AI sites of this pGEX-4T1 construct yielding pGEX

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GRIP1 563-767His6. Mutants of GRIP1 563-767 were generated by PCR or single stranded mutagenesis using oligonucleotides carrying the mutations and a pSG5 GRIP1 vector as template. The mutations were confirmed by sequence analysis and integrated into pGEX GRIP1 563-767His6 as NgoMI - Xho I fragments. The GRIP1 563-767 His6 GST fusion protein was expressed in HB101 at 37°C. Protein expression was induced with 1mM IPTG at an optical density (600 nm) of 0.7 and extended for 4 hours after induction. Cells were harvested by centrifugation, resuspended in sonication buffer (20mM TrisHCl pH 8.0, 0.1M NaCl, 10%glycerol, 0.1mM PMSF and protease inhibitors (Complete, EDTA free, Boehringer Mannheim)). The resuspended cells were freezethawed once, incubated on ice with 0.1mg/ml lysozyme for 20 minutes and lysed per sonication. The lysate was cleared by ultracentrifugation (Ti 45, 36000rpm, 1h 4°C), the supernatant filtered (Costar 0.2µm top filter) and loaded on a Talon column (Clontech). The column was washed with 10 column volumes of sonication buffer supplemented with 12mM imidazole and eluted with an imidazole gradient [12 - 100mM]. At this step the fusion proteins are about 95% pure. Imidazole was removed by gelfiltration on NAP columns (Pharmacia), and protein concentrations determined using the Biorad protein assay. Equal concentrations of the different derivatives of the fusion fragment were incubated with glutathione agarose (1h, 4°C) which was equilibrated in binding buffer (sonication buffer supplemented with 1mM DTT, 1mM EDTA and 0.01% NP-40). Beads were washed with at least 20 volumes of this buffer, diluted in binding buffer with 20% glycerol to 40%, frozen in aliques and stored at -70°C.

H. Coactivator GRIP1 563-767 His6

GRIP1 563-767 was cloned as a Bam HI - Xho I fragment derived fron pGEX GRIP1 563-767His6 into corresponding cloning sites of pET23a yielding pETGRIP1 563-767His6. The fragment was expressed in BL21DE3. Expression, cell lysis and Talon purification was identical as described for GST GRIP1 563-767His6. The protein eluted from a Talon column in two fractions, one at 12mM and one between 40 and 70mM imidazole. In the earlier eluting fraction the fragment was associated with a 70 kDa protein which was removed by a MonoQ run in 50mM TrisHCl pH7.5, 10% glycerol, 1mM EDTA, 1mM DTT, 0.1mM PMSF and protease inhibitors. GRIP1 563-767His6 eluted in the flow through and was concentrated by ultrafiltration. At this step the protein was more than 95% pure.

Example 2: Peptide synthesis

Coactivator peptides were obtained using standard techniques. All peptides were HPLC purified and analyzed by mass spectroscopy. Peptide concentrations were either determined

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spectroscopically using the tyrosine signal ($A_{276} = 1450$ M-1cm-1) or by amino acid analysis following standard techniques.

Example 3: Binding assays with nuclear receptors and coactivators

A. GST-GRIP Pull-down Assays and Peptide Competition Assays

Binding experiments were performed by mixing glutathione beads containing 10 μ g of GST fusion proteins (Coomassie Plus Protein Assay Reagent, Pierce) with 1-2 μ l of the [35 S]-labeled wild-type or mutant hTR β 1 (25 fmoles, 4000 cpm of receptor), or coactivators in 150 μ l of binding buffer (20 mM HEPES, 150 mM KCl, 25 mM MgCl₂, 10% glycerol, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and protease inhibitors) containing 2 mg/ml BSA for 1.5 hrs in the presence or absence of 1 μ M T₃. Beads were washed 3 times with 1 ml of binding buffer and the bound proteins were separated using 10% SDS-PAGE and visualized by autoradiography. Binding was quantitated by phosphorimaging using ImageQuant (Molecular Dynamics).

For in vitro binding studies GR, TR and their derivatives were translated in the presence of [35S]methionine using the TNT Coupled Reticulocyte System (Promega). Separate translations were performed in the presence and absence of $10\mu M$ dexamethasone or $1\mu M$ RU486 for GR and $10\mu M$ triiodothyronine for TR. Expression was quantified by phosphoimager analysis (BAS2000, Fuji). For all binding assays 50µl of a 20% bead suspension containing either 1.6 or 4.0 µM bound purified GST GRIP1 fragment (either 568-767 or 563-1121) was incubated with 0.2µl or 1.4µl in vitro transcribed and translated TR or GR, respectively. Binding was performed in the binding buffer described above supplemented with 20 µg/ml BSA and appropriate hormone. The chosen GST GRIP1 fragment concentrations were sufficient to bind either 70 or 100% of the TR derivatives. The reaction was incubated at 4°C under rotation for 2 hours. In case of competition experiments, the appropriate concentration of peptides were added to the reaction before addition of receptors. However, no differences in the results were noted by adding the peptides after half of the incubation of the GST GRIP1 fragment with nuclear receptors. This demonstrates that equilibrium is reached under the chosen conditions. Beads were washed five times with 200µl binding buffer + BSA at 4°C before elution of the bound proteins in 20µl SDS loading buffer. Eluted beads and input labeled protein were subjected to SDS-PAGE. The fraction of bound nuclear receptors was determined by phosphoimager analysis.

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B. GST-hTRβ1 Pull-down Assays

Assay and analysis was performed as for Example 3A. *In vitro* binding of [³⁵S]-labeled full-length GRIP1, [³⁵S]-labeled full-length SRC-1a, and [³⁵S]-labeled full-length hRXRα, to GST-hTRβ1 wild-type (WT) and mutants was performed. Mutants V284R, K288A, I302R, L454R, and E457K all bound to hRXRα with an affinity equivalent to wild type hTR. All of these mutants showed decreased ability to bind GRIP1 and SRC-1a, as expected from the results of Example 3A. The same results were obtained when a GST-SRC1 construct including SRC-1a amino acids 381-882 was tested for binding of [³⁵S]-Met-labeled full-length hTRβ1 WT and mutants (data not shown).

C. GST-hERa LBD Pull-down Assays

The wild-type and mutant GST-hERα LBDs were expressed in BL21(DE3) cells. Total ligand binding activity was determined by a controlled pore glass bead assay (Greene, et al., *Mol. Endocrinol.* (1988) 2:714-726) and protein levels were monitored by western blotting with a monoclonal antibody to hERα (H222). Cleared extracts containing the GST- hERα LBDs were incubated in buffer alone (50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1 mM DTT, 0.5% NP-40 and a protease inhibitor cocktail) or with 1 μM of either DES or OHT for 1 hour at 4°C. Extract samples containing thirty pmol of GST-LBD were then incubated with 10 μl glutathione-Sepharose-4B beads (Pharmacia) for 1 hour at 4°C. Beads were washed five times with 20 mM HEPES, pH 7.4, 400 mM NaCl, and 0.05% NP-40. ³⁵S-labeled GRIP1 was synthesized by *in vitro* transcription and translation using the TNT Coupled Reticulocyte Lysate System (Promega) according to the manufacturer's instructions and pSG5-GRIP1 as the template. Immobilized GST-hERα LBDs were incubated for 2.5 hours with 2.5 μl aliquots of crude translation reaction mixture diluted in 300 μl of Tris-buffered saline (TBS). After five washes in TBS containing 0.05% NP-40, proteins were eluted by boiling the beads for 10 minutes in sample buffer. Bound ³⁵S-GRIP1 was quantitated by fluorography following SDS-PAGE.

D. Electrophoretic Mobility Shift Assays

GRIP1, a mouse p160 coactivator, recognizes the ER α LBD in a ligand-dependent manner. The binding of agonists to the ER α LBD promotes recruitment of GRIP1, whereas binding of antagonists prevents this interaction (Norris, et al., *J. Biol. Chem.* (1998) 273:6679-88). While

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agonist-bound receptor will bind to all three of the NR boxes from GRIP1, ERα strongly prefers NR-box 2 (Ding, et al., *Mol. Endocrinol.* (1998) 12:302-13).

An electrophoretic mobility shift assay was used to directly assess the ability of the NR-box 2 peptide to bind the purified ER α LBD in the presence of either DES or OHT. Eight microgram samples of purified hER α -LBD bound to either DES or OHT were incubated in the absence of the peptide, i.e., buffer alone, or in the presence of either a 2-fold or 10-fold molar excess of the GRIP1 NR-box 2 peptide. The binding reactions were performed on ice for 45 minutes in 10 μ l of buffer containing 20mM Tris, pH 8.1, 1mM DTT, and 200mM NaCl and then subjected to 6% native PAGE. Gels were stained with GELCODE Blue Stain reagent (Pierce).

In the presence of the NR-box 2 peptide, the migration of the DES-hER α -LBD complex was retarded. In contrast, peptide addition had no effect on the mobility of the OHT-hER α -LBD complex. Hence, this peptide fragment of GRIP1 possesses the ligand-dependent receptor binding activity characteristic of the full-length protein.

Example: 4 Transfection assays with TR and hER α

HeLa cell transfection and assay conditions are described (Webb et al., *Mol Endocrinol* (1995) 9:443). For TR assays, 5 µg of the reporter p(DR-4)₂ -TK-LUC consisting of two copies of the DR-4 element (a direct repeat of the consensus TR response element (TRE) spaced by 4 base pairs) placed upstream of a minimal (-32/+45) thymidine kinase gene promoter linked to luciferase (LUC) coding sequences were used. A reporter containing palindromic TREs gave the same results (data not shown). Also, 2 µg of the hTR β 1 expression vector, pCMX-TR (WT or mutant), and 0.5 µg transfection control vector, pJ3LacZ, which contains the SV40 promoter linked to the β -galactosidase gene, were used. Other cells co-transfected with vector or receptor constructs can be used for same purpose. Alternative cells expressing sufficient levels of an endogenous receptor(s), or cells selected that express a single reporter, can be used for transfection assays, including MCF-7 cells expressing ER (Webb et al., *supra*) , and GC cells expressing TR (Norman et al., *J. Biol. Chem.* (1989) 264:12063-12073).

For hER α assays, 5 µg of estrogen responsive reporter plasmid encoding chloramphenicol acetyltransferase (CAT), pERE-collTATA (Sadovsky, et al., Mol Cell Biol. (1995) 15:1554), 0.5 µg expression vector encoding full-length hER α , pSG5-er HEGO (WT or mutants), and 2 µg of pj3lacz, were used. For the experiments of **Figures 2** and **4**, 0.5 µg of a full-length GRIP1 expression vector, pSG5-GRIP1, was also included in the transfection. Transfected cells were

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treated with or without 1 μ M T₃ or E₂, as indicated. After culturing for 24 hrs, the LUC or CAT activities were assayed and the β -galactosidase activities were also assayed to correct for differences in transfection efficiencies. The triplicate points were averaged and standard deviations were less than 10%.

Example 5: Hormone binding assays for wild-type and mutant TRs

The T₃ binding affinity constants (Kd) for *in vitro* -translated WT and mutant TRs were measured using [¹²⁵I] 3,5,3' triiodo-L-thyronine ([¹²⁵I]T₃) in gel filtration binding assays as described (Apriletti et al., *Protein Expr. Purif.* (1995) 6:363). Both the Kd and standard error (S.E.) values were calculated using the Prism computer program (GraphPad Software, Inc.). Mutations are indicated by the single-letter amino acid abbreviations, with the native residue name, followed by the primary sequence position number, and then the mutated residue name. The affinity of the WT TR is 81 ± 12 pM. The relative affinity was determined by dividing the WT Kd by each mutant Kd. The 37 mutants tested with their relative affinities are: E217R (123%), E227R (109%), K242E (92%), E267R (117%), H271R (123%), T277R (7%), T281R (145%), V284R (105%), D285A (89%), K288A (98%), C294K (94%), E295R (118%), C298A (87%), C298R (141%), E299A (171%), I302A (86%), I302R (99%), K306A (6%), K306E (6%), P384R (164%), A387R (107%), E390R (151%), E393R (146%), L400R (95%), H413R (109%), H416R (153%), M423R (156%), R429A (48%), S437R (170%), L440R (174%), V444R (89%), T448R (234%), E449R (36%), P453E (32%), L454R (26%), L456R (46%), E457K (71%).

Example 6: Coactivator binding assays for wild-type and mutant TRs

Wild type (WT) TR and most of the TR mutants liganded to 3,5,3'-triiodo-L-thyronine (T₃) bind equally well to the coactivator, GRIP1. In all cases, GRIP1 binding was hormone-dependent (data not shown). Mutations L454R and E457K in surface residues of helix 12 abolish GRIP1 binding (**Figure 1**). Mutations in two residues of helix 3, V284R and K288A, and two residues of helix 5, I302R and K306A, also impair binding (**Figure 1**). Five mutations with diminished GRIP1 binding (V284R, K288A, I302R, L454R, and E457K) also show decreased binding to another coactivator, SRC-1a (data not shown). Thus, these results show that two different coactivators recognize the same TR surface residues.

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5 Example 7: TR residues involved in ligand-dependent transcription activation in context of a cell

Residues involved in ligand-mediated transcription activation were identified by testing the TR mutants of Example 8 in HeLa cells. T₃ increased reporter gene activity 5-fold in cells expressing either WT TR or mutated TRs showing normal GRIP1 binding (representative mutants are shown in **Figure 1**. By contrast, TR mutants with diminished or absent GRIP1 binding (V284R, K288A, I302R, K306A, L454R, and E457K) show a diminished or absent response to T₃ which correlates with the GRIP1 binding defect. Overexpression of GRIP1 increases activation by the WT TR and rescues activation by TR mutants roughly in proportion to the severity of the defect of GRIP1 binding and activation (**Figure 2**). These results suggest that the same residues are required for coactivator binding, function of the endogenous coactivator(s) in HeLa cells, and responsiveness of TRs to GRIP1.

Example 8: Effect of TR mutations on other receptor functions

The effects of the mutations on other receptor functions also were examined. All of the mutants bound radiolabeled thyroid hormone (Kd values, 6%-234% that for native receptor); occasional lower values were expected because some residues have partially buried side chains. None of the residues that decrease GRIP1 binding affected TR binding to a GST-RXR fusion protein or to DNA using three different DNA half-site arrangements and testing with or without added RXR (data not shown). Some mutations that affect GRIP1 binding occur in a region spanning helices 3-5, which has been suggested as important for TR/RXR heterodimerization (O'Donnell et al., *supra*; Lee et al., *Mol. Endocrinol.* (1992) 6:1867-1873). In contrast, however, the above results indicate that these residues do not contribute to TR/RXR heterodimerization. Further, TRs mutated in the CBS residues retain the ability of WT TR of T₃—dependent inhibition of the activity of the Jun and Fos transcription factors at an AP-1 site (Saatcioglu et al., *supra*), suggesting that the CBS residues do not participate in TR actions mediated through these proteins. These data indicate that the mutational effects are specific, the amount of input labeled TR in the different reactions is comparable, and the levels of expression of the mutant TRs are comparable to those of WT receptors.

Example 9: Coactivator binding site in ER

Three separate mutations (K362A, V376R, and E542K) were created in human estrogen receptor- α (hER α) which align to three of the effective positions in hTR β 1 (K288A, I302R, and

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E457K). All three mutations diminish GRIP1 binding and abolish transcriptional activation (Figure 3), and mutant V376R, with 10% residual GRIP1 binding, was rescued partially by overexpression of GRIP1 (Figure 4). As a control, the ER mutants demonstrated a normal hormone-dependent ability to activate a vitellogenin-LUC hybrid reporter gene, GL45, which responds to the ER amino-terminal activation function (Berry et al., EMBO J (1990) 9:2811-2818) (data not shown). The finding that similar residues are required for GRIP1 binding and transcription activation activity in the TR and ER suggests that the coactivator binding site residues are similar in different nuclear receptors.

Example 10: Coactivator NR-box binding affinity for TR

To study the interaction between nuclear receptors and GRIP1 *in vitro*, a fragment of GRIP1 (563-767) was purified that contains all three NR-boxes (**Figures 6** and **7**). The fragment was found to be highly soluble and, in agreement with a secondary structure prediction using PhD, displays a mainly alpha-helical far UV-CD spectrum (data not shown). Three of the four helices predicted for the fragment include the NR-boxes at their C-terminus, suggesting that these boxes are part of amphipathic alpha-helices. These results show that the NR-boxes of GRIP1 are contained in a soluble, alpha-helical 24kD fragment.

Binding assays show that GRIP1 NR-boxes 1, 2 and 3, interact differentially with hTRß LBD (**Figure 7**). A GST-fusion of the GRIP1 (563-767) fragment strongly binds TR (kD or EC50) in a ligand depend fashion. Replacement of the hydrophobic residues of NR-box 3 with alanine does not reduce binding of TR significantly, whereas similar replacement of NR-box 2 results in loss of TR binding of about 50%. By titrating the amount of GRIP1 fragment, about a 4-fold stronger binding of TR for NR-box 2 (EC50 = $1.0~\mu$ M) over NR-box 3 (EC50 = $4.0~\mu$ M) was estimated. In the absence of functional NR-boxes 2 and 3, almost no binding to TR was detected suggesting that under these experimental conditions NR-box 1 is not a cognate binding site for TR. Full length TR or TR-LBD bound GRIP1 equally. These results show that TR recognizes GRIP1 NR-box 2 and 3, with preference for NR-box 2.

Example 11: Coactivator NR-box binding affinity for GR

GR also was found to bind GRIP1 (563-767) in a ligand-dependent manner (**Figure 8**). However, in contrast to TR, extension of GRIP1 (563-767) to residue 1121 increases binding to GR about 3-fold suggesting an additional binding site on GRIP1 for GR. Binding of the larger fragment remains ligand-dependent; no interaction can be observed in the presence of the GR partial

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antagonist RU486. These results are in agreement with *in vivo* 2-hybrid GR GRIP1 interaction studies. In the presence of ligand no difference was detected in the binding of GRIP1 by full length GR or a deletion mutant of GR that lacks the N-terminal activation domain AF-1. However in the absence of ligand, binding of GR to GRIP1 (563-1121) increased by about 10-fold indicating that sequences in the GR N-terminus are able to suppress binding of unliganded GR to this additional binding site in GRIP1. Additionally, GR did not bind to a GRIP1 (563-767) mutant in which both NR-box 2 and 3 are replaced by alanines, and binds most strongly to a fragment that lacks a functional NR-box 2. As with TR, GR does not recognize NR-box 1. In contrast to TR, the GR prefers NR-box 3 to NR-box 2. These results demonstrate that GR prefers binding to NR-box 3 and interacts with an additional GRIP1 site within the CREB (cAMP - response - element binding protein) - binding protein (CBP) binding site.

Example 12: Coactivator peptide binding affinity for TR

To investigate whether the preference of TR for NR-box 2 is dependent on the sequence or structural context of the NR-boxes, competition studies on the interaction of GRIP1 with hTRß LBD were performed using coactivator peptides containing different NR- boxes (NR-box 2 peptide (residues 11-23 of SEQ ID NO: 6) EKHKILHRLLQDS, and NR-box 3 peptide (residues 9-21 of SEQ ID NO: 7) ENALLRYLLDKDD) (**Figure 9**). Consistent with the interaction of hTR LBDß with GRIP1 (563-767) NR-box mutants, a peptide containing NR-box 1 competes the interaction of GRIP1 with hTRß LBD only at very high concentrations (EC50 = 130 μ M). Peptides containing either NR-box 2 or 3 compete GRIP1 (563-767) efficiently and display the preference of hTRß LBD for NR-box 2 (EC50 (NR-box 2) = 1.5 μ M, EC50 (NR-box 3) = 4 μ M). The apparent affinities (EC50) for peptides of NR-box 2 and 3 are comparable with the analogous GRIP1 (563-767) NR-box mutants suggesting that the preference of TR for NR-boxes is solely dependent on the sequence and independent of the structural context of the NR-boxes.

Peptides of NR-box 2 or 3 compete GRIP1 (563-767) containing functional NR-boxes 2 and 3 or a mutant of this fragment that contains only a functional NR-box 2 with comparable affinity. Thus, while TR can bind both NR-box 2 and 3, in a GRIP1 coactivator peptide fragment containing both boxes, TR preferentially binds NR-box 2.

These results show the preference of TR for NR-box 2 is sequence dependent.

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The same types of assays for TR competition are performed to assess coactivator peptide binding affinity for GR. The peptide concentrations are normalized relative to TR for obtaining comparable dose response curves.

Example 13: Binding affinity of TR for extended coactivator peptides

Sequence identity between all three central NR-boxes of the p160 coactivator family is limited to the conserved leucine residues of the (SEQ ID NO: 1) LxxLL motif (**Figure 6**). However, the sequence conservation of a particular NR-box can extend into neighboring residues. To investigate the contribution of these neighboring residues to affinity and specificity of the different NR-boxes for TR, the ability of peptides containing individual NR-boxes with different lengths of adjacent sequences to compete with the interaction of GRIP1 (563-767) with hTRß LBD were compared (**Figure 10**).

A peptide consisting of the minimal motif of NR-box 3 (residues 12-17 of SEQ ID NO: 7; LLRYLL) does not compete the TR LBD interaction with GRIP1 (563-767). A peptide consisting of the NR-box 2 (residues 15-20 of SEQ ID NO: 6; ILHRLL) also does not sufficiently compete the interaction (data not shown). Extending peptides containing a (SEQ ID NO: 1) LxxLL motif to include adjacent residues increased affinity for both NR-box motifs and magnified the preference of TR for NR-box 2 (NR-box 2 peptides: (residues 11-23 SEQ ID NO: 6) EKHKILHRLLQDS and (residues 7-23 of SEQ ID NO: 6) TSLKEKHKILHRLLQDS; and NR-box 3 peptides: (residues 8-24 of SEQ ID NO: 7) KENALLRYLLDKDDTKD and (residues 5-24 of SEQ ID NO: 7) PKKKENALLRYLLDKDDTKD). A chimeric peptide containing the NR-box 3 motif in the context of the NR-box 2 flanking sequences (SEQ ID NO: 31; TSLKEKHKLLRYLLQDSS) binds like a NR-box 2 peptide.

These results demonstrates that preference of TR for NR-box 2 is at least partially due to features of the bound peptide (residues 15-20 of SEQ ID NO: 6; ILHRLL), but that their affinity and specificity is modulated by adjacent sequences.

Example 14: Binding affinity of TR and GR for mutant coactivator

A. TR affinity for ILxxLL motif residues

To investigate the role of the hydrophobic residues in NR-box 2, individual residues of the (residues 15-20 of SEQ ID NO: 6) ILHRLL motif were replaced by alanine in the background of GRIP1 (563-767) containing a non-functional NR-box 3 (**Figure 11**). Surprisingly, replacement of

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any of the conserved leucines prevents binding to TR almost completely. Only replacement of the nonconserved isoleucine exhibited a lessened but still severe impact on the affinity of NR-box 2 for TR. As replacement of a single leucine by alanine is sufficient to overcome the interaction of both the remaining hydrophobic residues and adjacent sequences with hTR\$\beta\$ LBD, it appears that their contribution to the affinity of NR-box 2 for hTR\$\beta\$ LBD is cooperative rather than additive.

Similar results were obtained by competing the interaction of hTRß LBD with the GRIP1 (563-767) NR-box 3 mutant using peptides in which either IL, HR or LL of the NR-box 2 motif are replaced by alanines (**Figure 11**). Whereas the peptides containing the IL or LL replacement failed to interact with the hTRß LBD even at very high concentrations, in agreement with a proposed alpha-helical structure of the motif, replacement of the "HR spacer" by alanines showed a marginal effect on the affinity of the peptide for TR-LBD.

Replacement of single leucine residues of NR-box 2 by phenylalanine reduced the affinity of NR-box 2 peptides for TR LBD about 100-fold, replacement of the isoleucine about 10-fold (**Figure 11**). Therefore, the interaction of TR with GRIP1 relies not simply on the hydrophobicity of the (SEQ ID NO: 1) LxxLL motif, but also on positive contributions by the leucine residues themselves.

These results demonstrate that single mutations of the conserved leucines in the (SEQ ID NO: 1) LxxLL motif strongly reduce affinity of GRIP1 for hTRß LBD.

Collectively, the above examples demonstrate that peptides containing NR-boxes, particularly NR-box 2, reproduce the affinity and specificity of the interaction of GRIP1 (563-767) with hTRß LBD.

B. TR affinity of FxxLW and FxxAL motif residues

The three conserved leucines of the NR-box 2 (SEQ ID NO: 2) ILxxLL motif are embedded in the hydrophobic cleft of the hTRß LBD:NR-box 2 interaction surface, whereas the non conserved isoleucine is located on the rim of this cleft where structural changes can be more easily accommodated (See Example 18). In agreement with this structure, replacement of this residue by alanine or phenylalanine reduced binding to hTRß LBD to a less extent than the comparable mutations of the conserved leucine residues. The surface generated by the three conserved leucines (L690, L693, L694) of the NR-box 2 peptide (residues 12-24 of SEQ ID NO: 6) 686-KHKILHRLLQDSS-698 is highly complementary to the corresponding binding site in the hTRß LBD (Figures 16 and 17). Comparison of this binding site to other nuclear receptors shows that it

contains a structural motif that is unique, highly conserved and present in all known structures of nuclear receptor LBDs (Wurtz et al., *Nat Struct Biol.* (1996) 3:87-94; Wagner et al., *supra*; Renaud et al., *Nature* (1995) 378:681-689; Bourguet et al., *Nature* (1995) 375:377-382; and Brzozowski et al., *Nature* (1997) 389:753-758).

Interaction of highly conserved hydrophobic motifs, which are part of amphipathic alphahelices, with complementary hydrophobic surfaces resembles a feature observed for the interaction of several other transcriptional activators with their target proteins (p53:MDM2, VP16:TAFII31 or CREB:KIX-CBP). However, the motifs of p53 (FxxLW), VP16 (FxxAL) and CREB (YxxIL) differ from the (SEQ ID NO: 1) LxxLL motif of nuclear receptor coactivators. A Fxxxh motif may be generally involved in interaction with TAFII31, where "h" represents any hydrophobic residue. Though with respect to the known structures, complementarity of the interacting hydrophobic surfaces identified here seem to be a common feature of these interactions, cross-reactions between different motifs are possible. For instance, VP16, p53, and p65 (FxxFL) are able to functionally interact with TAFII31, or p53 and E2F1-DP1 (FxxLL) both interact with MDM2. These interactions are sensitive to mutations in the Fxxxh motif. Therefore it appears that either complementarity of the hydrophobic surfaces is not an absolute requirement or that induced fitting of the interacting surfaces is possible.

Based on these observations, studies were performed to determine whether GRIP1 interacts with TAFII31 or MDM2. However, no interaction was detected. GRIP1 mutants changing NR-box 2 (SEQ ID NO: 1; LxxLL) to VP16 (SEQ ID NO: 4; FxxAL) or p53 (SEQ ID NO: 3; FxxLW) like binding sites also failed to bind TAFII31 or MDM2 demonstrating that the presence of the correct binding site is not sufficient to create binding (data not shown). Moreover, peptides containing the VP16 or p53 binding sites are not able to compete the interaction of GRIP1 with TR, even in very high concentration, but do compete the interaction with GR (data not shown). The affinity of this interaction is weak, but comparable to affinity of a peptide of NR-box 2 that, in the context of a GRIP1 mutant lacking NR-box 3, binds GR *in vivo* (Ding et al., *supra*). This binding is only about ten times less than a peptide containing NR-box 3, GR's primary binding site.

As shown above, GR binds GRIP1 (563-767) with about one-fifth the affinity than a comparable amount of TR. Thus, the high concentration of NR-box 3 peptide required to compete the interaction of GR with GRIP1 (563-767) may rather reflect a weak affinity of GR for the peptide rather than a particular strong interaction of GR with GRIP1 (563-767).

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These results suggest that at least on the peptide level, other hydrophobic motifs besides (SEQ ID NO: 1) LxxLL can interact with the coactivator binding site, but that it is receptor dependent.

C. TR affinity for residues adjacent to ILxxLL motif

Peptides containing a FxxLL motif bind TR but with two orders of magnitude lower affinity than a (SEQ ID NO: 1) LxxLL motif (**Figure 11**). To test whether the additional changes in the hydrophobic motif or adjacent sequences of the VP16 peptide prevent its binding to TR, a chimeric peptide containing the NR box-2 motif (SEQ ID NO: 1) LxxLL in the context of the VP16 sequence was constructed. This peptide binds to TR but with an about 100-fold lower affinity than the original NR-box 2 peptide. Thus, the inability to bind the VP16 peptide appears to be due to the combination of an imperfect hydrophobic motif and the incompatibility of TR to adjacent sequences of the VP16 motif.

As the interaction of the chimeric peptide with GR was comparable to the original NR-box 2 and VP16 peptides, this incompatibility appears due to TR-specific features in the NR-box interaction surface. These results show sequences adjacent the NR-box motif LxxLL can reduce binding of NR-box 2 to TR, but not GR.

Example 15: Crystallization and Structure Determination of NR LBD Complexes

A. Crystallization of hTRB LBD with T₃ and GRIP1 NR-box 2 Peptide

Several peptides containing GRIP1 NR-box 2 were tested in crystallization trials with the hTRß LBD. The complex of the hTRß LBD with the GRIP1 NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) produced crystals that were dependent on both the presence and the concentration of the peptide. Without the peptide, the hTRß LBD precipitated immediately. However, nucleation was erratic, but could be overcome through seeding of prepared drops with microcrystals of the hTRß LBD:GRIP1 NR-box 2 peptide complex. Structure of the hTRß LBD:GRIP1 NR-box 2 peptide complex was determined by molecular replacement using the structure of the hTRß LBD determined previously (Wagner et al., *supra*), and refined to a resolution of 3.6Å (**Table 1**). The refined model consists of residues K211-P254 and V264-D461 of monomer 1 of the hTRß LBD, residues K211-P254 and G261-D461 of monomer 2 of the hTRß LBD, and the GRIP1 NR-box 2 peptides (residues 14-24 of SEQ ID NO: 6) 688-

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5 KILHRLLQDSS-698, and (residues 14-22 of SEQ ID NO: 6) 688-KILHRLLQD-696 (Appendix 1).

Briefly, the complex between the hTRß LBD and the GRIP1 NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) was prepared by mixing (equal) volumes of a solution of 9mg/ml hTRß LBD in 20mM HEPES pH 7.4 with a solution of 14 mM GRIP1 in 0.4mM ammonium acetate pH 4.72, and incubating the mixture on ice for 1 hour. Crystals were obtained after 2 days at 4°C using hanging drop vapor diffusion from a drop containing 1.5μl of hTRß LBD:GRIP1 complex, prepared as described, and 0.5μl 15%PEG 4K, 0.2M sodium citrate pH 4.9, suspended above a reservoir containing 10% PEG 4K, 0.1M ammonium acetate, and 0.05 M sodium citrate (pH 5.6). After allowing the drop to equilibrate for 1 hour, 0.2μl of 10-3 to 10-5 dilutions of microcrystals in reservoir buffer were introduced to provide nucleation. Crystals are of space group P3121 (a=95.2, b=95.2, c=137.6) and contain two molecules of the hTRß LBD and two molecules of the GRIP1 NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6).

Table 1

Data collection, phasing, and refinement statistics

			Da	ata collection	1	
Data set	Resolution (Å)		Reflections		Coverage (%)	$R_{ ext{sym}}$
Native	3.6		measured 35565	unique 8490	96.3	0.007
			Ro	tation searc	h	
Search model	Euler angles (°)				Correlation coefficient	
	Θ_1		Θ_2	Θ_3	Highest peak	Highest false peal
hTR β LBD	M1	60.12		241.90	16.3	
	M2	9.93	87.70	180.6	15.9	14.2
·-··			Tra	nslation sear	rch	
	Fractional coordinates			ordinates	Translation function	
	İ	X	у	Z	Highest peak (o)	Highest false peak (o)
	M1	0.522	0.428	3 0.250	19.52	10.02
	M2	0.200	0.932	0.119	26.11	5.77
				Refinement		
	Resolution (Å)		Reflection		R	R _{free}
F > 2(25 - 3.7		7614		0.2990	0.3219
All data	25 - 3.7		7851		0.3010	0.317

 $R_{\text{sym}} = \Sigma_h \Sigma_i \mid I_{h,i} \hat{\mathbf{u}} \left(I_h(\mid / \Sigma I_h \text{ for the intensity } (I) \text{ of } i \text{ observations of reflection } h.$ Correlation coefficient = $\Sigma_h Eo^2 Ec^2 - Eo^2 Ec^2 / \left[\Sigma_h \left(Eo_2 - Eo^2 \right)^2 \Sigma_h \left(Ec^2 - Ec^2 \right)^2 \right]^{1/2}$ Translation function $(t_a, t_b, ...) = \Sigma_h \left(|Eo_{(h)}|^2 - \Sigma_h < |Eo_{(h)}|^2 > \right) \left(Ec_{(h, t_a, t_b, ...)} \right)^2 - < |Ec_{(h)}|^2$

where E_0 represents the normalized observed structure factor amplitudes, and E_c represents the normalized structure factors for the search model in a triclinic unit cell with dimensions identical to that of the crystal. The reported peak height represents the value of the function for the translation (t_a, t_b) of the NCS monomers, divided by the rms value of the translation function density.

R factor = $\Sigma | F_{\text{obs}} - F_{\text{calc}}| / \Sigma | F_{\text{obs}}|$.

 R_{free} is calculated the same as R factor, except only for 10% of the reflections that were set aside for cross validation and not used in refinement.

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B. Crystallization of hERα LBD with DES and GRIP1 NR-box 2 Peptide

Crystals of a DES-hER\alpha LBD-GRIP1 NR-box 2 peptide complex were obtained by hanging drop vapor diffusion. Prior to crystallization, the DES-hERa LBD (residues 297-554) complex was incubated with a 2-4 fold molar excess of the GRIP1 NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) for 7-16 hr. Two μL samples of this solution were mixed with equal volume samples of reservoir buffer consisting of 25-27% (w/v) PEG 4000, 90 mM Tris (pH 8.75-9.0) and 180 mM Na Acetate and suspended over wells containing 800 μL of the reservoir buffer. After 4-7 days at 19-21°C, rod-like crystals were obtained. The coactivator complex crystals lie in the spacegroup P2₁ with cell dimensions a=54.09, b=82.22, c=58.04 and β =111.34. Two molecules each of the DES-LBD and the coactivator peptide form the asymmetric unit. A 200 um x 40 um x 40 um crystal was transferred to a cryosolvent solution containing 25% (w/v) PEG 4000, 10% (w/v) ethylene glycol, 100 mM Tris (pH 8.5), 200 mM Na Acetate and 10 µM peptide and frozen in an N2 stream at -170°C in a rayon loop. Diffraction data from this crystal were measured at -170°C using a 300 mm MAR image plate at the Stanford Synchrotron Radiation Laboratory (SSRL) at beamline 7-1 at a wavelength of 1.08 Å. The diffraction images were processed with DENZO and scaled with SCALEPACK (Otwinowski, et al., Methods Enzymol. (1997) 276:307-326) using the default -3σ cutoff.

C. Crystallization of hERα LBD with OHT

Crystals of the hER α LBD (residues 297-554) complexed to OHT were obtained by the hanging drop vapor diffusion method. Equal volume aliquots (2 μ L) of a solution containing 3.9 mg/mL protein-ligand complex and the reservoir solution containing 9% (w/v) PEG 8000, 6% (w/v) ethylene glycol, 50 mM HEPES (pH 6.7) and 200 mM NaCl were mixed and suspended over 800 μ L of the reservoir solution. Hexagonal plate-like crystals formed after 4-7 days at 21-23°C. Both crystal size and quality were improved through microseeding techniques. These crystals belong to the space group P6522 with cell parameters a=b=58.24 Å and c=277.47 Å. The asymmetric unit consists of a single hER α LBD monomer; the dimer axis lies along a crystallographic two-fold. A single crystal (400 μ m x 250 μ m x 40 μ m) was briefly incubated in a cryoprotectant solution consisting of 10% (w/v) PEG 8000, 25% (w/v) ethylene glycol, 50 mM HEPES (pH 7.0) and 200 mM NaCl and then flash frozen in liquid N₂ suspended in a rayon loop. Diffraction data were measured at -170°C using a 345 mm MAR image plate at SSRL at beamline 9-1 and at a wavelength of 0.98 Å. The diffraction images were processed with DENZO and scaled with SCALEPACK (Otwinowski, et al., *supra*) using the default -3 σ cutoff.

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5 Example 16: Structure determination and refinement of NR LBD complexes

A. Structure of hTRß LBD with T₃ and GRIP1 NR-box 2 Peptide

Data were measured using Cu Ka radiation from an R-axis generator at 50 kV and 300 mA with a 0.3mM collimator and a Ni filter. Reflections were measured using an R-Axis II detector and integrated with Denzo, and equivalent reflections scaled using Scalepack (Otwinowski and Minor, "Processing of x-ray diffraction data collected in oscillation mode." In Macromolecular Crystallography, Part A (ed. C.W. Carter, Jr. and R.M. Sweet), pp. 307-326. Academic Press, New York, NY). Possible rotation function solutions were calculated using normalized amplitudes in AMORE from a model of hTRB LBD with the ligand, T3, omitted; translation function solutions were subsequently determined using TFFC for the two rotation solutions with the highest correlation coefficients. For two hTRB LBD molecules in the asymmetric unit, the calculated solvent content is 52%. After rigid body refinement of the two hTRB LBD molecules, electron density maps were calculated. Strong positive density present in both the anomalous and conventional difference Fourier maps for the iodine atoms of the T3 ligand confirmed the correctness of the solution. The iodine atoms for both T3 ligands were modeled as a rigid body, and the structure refined with strict NCS symmetry using CNS. Both 2FoFc and FoFc electron density maps showed interpretable density, related by the NCS operator, near H12 of both molecules of the hTR β LBD. The electron density could be modeled as a short α -helix, and the observed side chain density was used to tentatively assign the sequence and direction to the chain. The refined model consists of residues of the hTRB LBD, and peptide residues of the GRIP1 NR-box 2 peptide 686-KHKILHRLLODSS-698 (residues 12-24 of SEQ ID NO: 6).

Atomic coordinates of the hTR3 LBD:GRP1 site 2 peptide complex are attached as Appendix 1.

B. Structure of hERα LBD with DES and GRIP1 NR-box 2 Peptide

Initial efforts to determine the structure of the DES-hERα LBD-NR box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) complex utilized a low resolution (3.1 Å) data set (data not shown). A self-rotation search implemented with POLARRFN ("The CCP4 suite: programs for protein crystallography", *Acta Crystallogr*. (1994) D50:760-763) indicated the presence of a noncrystallographic dyad. The two LBDs in the asymmetric were located by molecular replacement in AMoRe (CCP4, 1994) using a partial polyalanine model of the human RARγ LBD (Renaud, et al., *supra*) as the search probe (R=58.2%, CC=35.6% after placement of

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both monomers). Given that the model at this point was both inaccurate (r.m.s.d. 1.7 Å between this model and the final model based on Cα positions) and incomplete (accounting for only ~45% of the total scattering matter in the asymmetric unit), an aggressive density modification protocol was undertaken. Iterative cycles of two-fold NCS averaging in DM (CCP4, 1994) interspersed with model building in MOLOC (Muller, et al., *Bull. Soc. Chim. Belg.* (1988) 97:655-667) and model refinement in REFMAC (Murshudov, et al., *Acta Crystallogr.* (1997) D53:240-255) (using tight NCS restraints) were used to quickly build a model of the LBD alone. For this procedure, MAMA (Kleywegt, et al., "Halloween...masks and bones. In From First Map to Final Model", Bailey, et al, eds., Warrington, England, SERC Daresbury Laboratory, 1994) was used for all mask manipulations and PHASES (Furey, et al., PA33 *Am. Cryst. Assoc. Mtg. Abstr.* (1990) 18:73) and the CCP4 suite (CCP4, 1994) were used for the generation of structure factors and the calculation of weights.

However, although the DES-hERa LBD-NR complex model accounted for ~90% of the scattering matter in the asymmetric unit, refinement was being hampered by severe model bias. The high-resolution data set of the DES-hERa LBD-NR-box 2 peptide complex became available when the R_{free} of the OHT-hER α LBD model was ~31%. Both monomers in the asymmetric unit of the DES complex crystal were relocated using AMoRe and the incompletely refined OHT-hER α LBD model (with helix 12 and the loop between helices 11 and 12 removed) as the search model. The missing parts of the model were built and the rest of the model was corrected using MOLOC and two-fold averaged maps generated in DM. Initially, refinement was carried out with REFMAC using tight NCS restraints. At later stages, the model was refined without NCS restraints using the simulated annealing, minimization and B-factor refinement protocols in X-PLOR and a maximumlikelihood target. All B-factors were refined isotropically and anisotropic scaling and a bulk solvent correction were used. The R_{free} set contained a random sample of 6.5% of all data. In refinement, all data between 27 and 2.03 Å (with no σ cutoff) were used. The final model was composed of residues 305-549 of monomer A, residues 305-461 and 470-554 of monomer B, residues 687-697 of peptide A, residues 686-696 of peptide B, 164 waters, two carboxymethyl groups and a chloride ion. According to PROCHECK, 93.7% of all residues in the model were in the core regions of the Ramachandran plot and none were in the disallowed regions. Thus, the structure of the DES-hER α LBD-NR-box 2 peptide complex has been refined to a crystallographic R-factor of 19.9% (R_{free}=25.0%) using data to 2.03 Å resolution.

Ile 689 from the peptide interacts with three receptor residues (Asp 538, Glu 542 and Leu 539). The γ -carboxylate of Glu 542 forms hydrogen bonds to the amides of residues 689 and 690 of

the peptide. A water-mediated hydrogen bond network is formed between the imidazole ring of His 377, the γ-carboxylate of Glu 380, and the amide of Tyr 537. Three residues (Glu 380, Leu 536 and Tyr 537) interact with each other through van der Waals contacts and/or hydrogen bonds. Intriguingly, mutations in each these three residues dramatically increase the transcription activity of unliganded ERα LBD (Eng, et al., *Mol. Cell. Biol.* (1997) 17:4644-4653); Lazennec, et al., *Mol. Endocrinol.* (1997) 11:1375-86; White, et al., *EMBO J.* (1997) 16:1427-35). Atomic coordinates of DES-LBD-peptide complex are attached as **Appendix 2**.

Table 2 Summary of Crystallographic Statistics Ligand **OHT DES** 15 **Data Collection** P6522 $P2_1$ Space group 2.03 1.90 Resolution 104189 269253 Observations 30265 23064 Unique 99.1 98.4 20 Completeness (%) $R_{sym}(\%)^a$ 7.8 7.0 16.1 9.8 Average I/σI Refinement 4180 2070 Number of non-hydrogen atoms 25 R_{crvst} (%)^b/ R_{free} (%) 19.9/25.0 23.0/26.1 0.006 0.006 Bond r.m.s. deviation (Å) 1.05 Angle r.m.s. deviation (°) 1.05 40.4 Average B factor (Å²) 34.0 $R_{svm} = \sum_{i} |I_{i} - \langle I_{i} \rangle| / \sum_{i} I_{i}$ where $\langle I_{i} \rangle$ is the average intensity 30 over symmetry equivalents b $R_{cryst} = \sum |F_o - F_c| / \sum |F_o|$

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C. Structure of hERa LBD-OHT complex

The OHT complex data set was then collected. Starting with one of the monomers of the preliminary low-resolution DES-hERa LBD-NR-box 2 peptide model as the search probe, molecular replacement in AMoRe was used to search for the location of LBD in this crystal form in both P6₁22 and P6₅22. A translation search in P6₅22 yielded the correct solution (R=53.8%, CC=38.2%). In order to reduce model bias, DMMULTI (CCP4, 1994) was then used to project averaged density from the DES complex cell into the OHT complex cell. Using MOLOC, a model of the hERa LBD was built into the resulting density. The model was refined initially in REFMAC and later with the simulated annealing, positional and B-factor refinement protocols in X-PLOR (Brunger, X-PLOR Version 3.843, New Haven, Connecticut: Yale University, 1996) using a maximum-likelihood target (Adams, et al., Proc. Natl. Acad. Sci. USA (1997) 94:5018-23). Anisotropic scaling and a bulk solvent correction were used and all B-factors were refined isotropically. Except for the R_{free} set (a random sampling consisting of 8% of the data set), all data between 41 and 1.9 Å (with no σ cutoff) were included. The final model consisted of residues 306-551, the ligand and 78 waters. According to PROCHECK (CCP4, 1994), 91.6% of all residues in the model were in the core regions of the Ramachandran plot and none were in the disallowed regions. Thus, the structure of the OHT-hERa LBD complex has been refined against data of comparable resolution (1.90 Å) to a crystallographic R-factor of 23.0% (R_{free}=26.2%). Atomic coordinates of OHT-hERa LBD complex are attached as Appendix 3.

Example 17: Structural analysis of hTRß LBD:GRIP 1 NR-box 2 peptide complex

A. Structure of cocrystal complex (contents of asu)

The asymetric unit (asu) of the crystal contains two monomers of the hTR β LBD and two molecules of the GRIP1 NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6), which observes the NCS relation of the two TR monomers (**Figure 12**). The structure of the hTR β LBD, which closely resembles that of the rTR α LBD (Wagner et al., <u>supra</u>), consists of twelve alpha-helices and two β -strands organized in three layers, resembling an alpha-helical sandwich. The only significant difference between the hTR β LBD and the rTR α LBD is disorder in the loop between helices H1 and H3. The GRIP1 NR-box 2 peptide forms an amphipathic α -helix of about 3 turns, preceded by 2 residues and followed by 3 residues in extended coil conformation.

The relation of the two monomers of the hTRB LBD is primarily translational, and does not resemble the homodimer structures reported for the hRXR, or the hER (Bourguet et al., *supra*;

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Brzozowski et al., <u>supra</u>). Furthermore, the interface between the two monomers does not involve residues necessary for formation of the physiological TR dimer. Instead, one of the cocrystal peptides appears to bridge the interaction between the two monomers. The hydrophobic face of the alpha-helix of the cocrystal peptide contacts monomer 1 of the hTRß LBD at H3, H5, and H12, while the hydrophilic face contacts monomer 2 at the hairpin turn preceding strand S3. The second cocrystal peptide also contacts monomer 2 at H3, H5, and H12, and the two cocrystal peptides observe the same NCS relation as TR LBD monomers.

The common interface between both cocrystal peptides and the hTRß LBD buries the hydrophobic residues that define the cocrystal peptide (SEQ ID NO: 1) LxxLL sequence motif, residues Ile689, Leu690, Leu693, and Leu694; against the surface of the receptor LBD (**Figures 16 and 17**). The presence of the second peptide in the crystal, duplicating the interactions of the hydrophobic residues, suggests those interactions are specific and drive the interaction of the peptide with the hTRß LBD, while the hydrophilic interactions provide a fortuitous crystal contact and account for the dependence of crystallization on the presence and concentration of the peptide.

B. Structure of the GRIP1 NR-box 2 peptide

The GRIP1 NR-box 2 peptide used in the crystallization is 13 amino acids long (residues 12-24 of SEQ ID NO: 6; 686-KHKILHRLLQDSS-698). For the NR-box 2 peptide in monomer 1 (peptide 1), 12 amino acids are ordered in the crystal. Residues K688 - Q694 form an amphipathic helix, with residues K686-H687 and D695-S698 on either end in extended coil conformations. For the NR-box 2 peptide in monomer 2 (peptide 2), residues K688 - Q694 again form an amphipathic helix, but the ends of the peptide are disordered. While the resolution of the current data prevents absolute assignment of hydrogen bonds, it is evident from the periodicity of the side chain density that the central residues form an alpha-helix. In the absence of TR the far UV-CD spectrum of the GRIP1 NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) appears to be random coil (data not shown). Stable helix formation may thus be induced by the interaction of the hydrophobic amino acids with the receptor LBD as it has been proposed in other protein:protein interactions, such as p53:MDM2 (Kussie et al., *Science* (1996) 274:948-953), VP16:TAF31 (Uesugi et al., *Science* (1996) 277:1310-1313), and CREB:KIX-CBP (Radhakrishnan et al., *Cell* (1997) 91:741-752).

C. Structure of the hTRB LBD:GRIP1 NR-box 2 peptide interface

The hTRß LBD of the cocrystal contributes residues from three helices, H3, H5, and H12 to the interface, which pack against one another to create a hydrophobic cleft. The residues lining the cleft are I280, T281, V283, V284, A287, and K288 from H3; Q301, I302, L305, and K306 from H5; and L454, E457, V458, and F459 from H12. A cysteine residue (C309) from H6 appears to provide a partial surface that is buried deep within the bottom of the cleft.

The GRIP1 NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) binds at the junction of H3 and H12. Leu690 of the bound peptide inserts into a shallow but defined depression at the base of the cleft, making van der Waals contact with L454 and V458 of H12, while peptide residue Ile689 packs against L454 of H12 outside the edge of the cleft; L454, then, interdigitates between the two residues. One further turn C-terminal along the alpha-helix, L693 and L694 of the bound peptide pack into complementary pockets within the hydrophobic cleft. Peptide residue L693 forms van der Waals contact with V284 of H3, while peptide residue L694, bound more deeply in the cleft, makes contact with F298 and L305 of H4 and H5. The hydrophobic interactions of the GRIP1 NR-box 2 peptide with the hTRß LBD are observed for both cocrystal peptides 1 and 2 in their respective monomers of the crystal dimer complex, suggesting that the interactions are specific to the peptide, and not induced by crystallization.

Example 18: Overall Structure of the DES-hERα-LBD-NR-box 2 Peptide Complex

The asymmetric unit of the DES-hERα LBD-NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) complex crystals contains the same noncrystallographic dimer of LBDs that has been observed in the previously determined structures of the LBD bound to both E2 and RAL (Brzozowski, et al., *supra* and Tanenbaum, et al., *supra*). Beyond the flexible loops between helices 2 and 3 and helices 9 and 10, the two LBDs of the dimer adopt similar structures (r.m.s.d. 0.47 Å based on Cα positions). The conformation of each LBD complexed with DES closely resembles that of the LBD bound to E2 (Brzozowski, et al., *supra*); each monomer is a wedge shaped molecule consisting of three layers of eleven to twelve helices and a single beta hairpin. In each LBD, the hydrophobic face of helix 12 is packed against helices 3, 5/6 and 11 covering the ligand binding pocket. One NR-box 2 peptide is bound to each LBD in a hydrophobic cleft composed of residues from helices 3, 4, 5 and 12 and the turn between 3 and 4. The density for both peptides in the asymmetric unit is continuous and unambiguous. Residues 687 to 697 from peptide A and residues 686 to 696 from peptide B have been modeled; the remaining residues are disordered. Given that each peptide lies within a different environment within the crystal, it is

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striking that from residues Ile 689 to Gln 695 each peptide forms a two turn, amphipathic α helix. Flanking this region of common secondary structure, the peptides adopt dissimilar random coil conformations.

Example 19: Structure of the OHT-hERα LBD Complex

The binding of OHT induces a conformation of the hER α LBD that differs in both secondary and tertiary structural organization from that driven by DES binding. In the DES complex, the main chain from residues 339 to 341, 421 to 423, and 527 to 530 form parts of helices 3, 8 and 11 respectively. In contrast, these regions adopt an extended conformation in the OHT complex. In addition, the composition and orientation of helix 12 are different in the two structures. Helix 12 in the DES complex consists of residues 538 to 546 whereas helix 12 in the OHT complex consists of residues 536 to 544. Most dramatically, rather than covering the ligand binding pocket as it does in the DES complex, helix 12 in the OHT complex occupies the part of the coactivator binding groove formed by residues from helices 3, 4, and 5, and the turn connecting helices 3 and 4. This alternative conformation of helix 12 appears to be similar to that observed in the RAL complex (Brzozowski, et al., *supra*).

Example 20: Coactivator binding site structure and function

A. TR coactivator binding site

The above examples demonstrate that nuclear receptors, exemplified by TR, GR and ER, are recognized by specific coactivators that bind thereto through a coupling surface comprising a hydrophobic cleft and a charged hydrophobic perimeter. Identification and characterization of this coupling surface and the coactivator binding site of nuclear receptors offers a new target for the design and selection of compounds that modulate binding of coactivator to nuclear receptors.

Residues forming the coactivator binding site were found to cluster within a surprisingly small area with well-defined borders (see, e.g., **Figures 5, 14,** and **15**). As is shown in above Examples, mutated residues nearby this area do not affect coactivator binding or transcriptional activation. Additionally, the coactivator binding assays and structural analyses demonstrated that NR-box containing proteins and peptides bind to this site. These results also showed that the GRIP1 coactivator protein binds to the site through a highly (SEQ ID NO: 1) LxxLL.

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The structural analyses showed that residues contacting a conserved leucine residue of the (SEQ ID NO: 1) LxxLL motif included V284, F293, I302, L305 and L454. Residues within 4.5Å of an atom of the bound peptide included T281, V284, K288, F293, Q301, I302, L305, K306, P453, L454 and E457. Structural analyses also revealed two other features of the site: a hydrophobic residue from helix 12 (Phe459) that contributes to local packing, and a cysteine residue contributed by helix 6 (Cys309) that provides a partial surface buried deep within the site. Mutational analyses showed that residues which block GRIP1 and SRC-1 coactivator binding when mutated are residues V284, K288, I302, K306, L454, and V458. Mutated residues likely to undergo a conformational change upon hormone binding included Leu454 and Glu457. Thus, the site identified by mutational, binding assays and crystallography corresponds to a surprisingly small cluster of residues on the surface of the LBD that define a prominent hydrophobic cleft formed by hydrophobic residues corresponding to human TR residues of C-terminal helix 3 (Ile280, Val283, Val284, and Ala287), helix 4 (Phe293), helix 5 (Ile302 and Leu305), helix 6 (Cys309), and helix 12 (Leu454, Val458 and Phe459). Collectively, the Examples indicate that residues forming the site are amino acids corresponding to human TR residues of C-terminal helix 3 (Ile280, Thr281, Val283, Val284, Ala287, and Lys288), helix 4 (Phe293), helix 5 (Gln301, Ile302, Leu305, Lys306), helix 6 (Cys309), and helix 12 (Pro453, Leu454, Glu457, Val458 and Phe459). The coactivator binding site is highly conserved among the nuclear receptor super family (Figure 19).

The coactivator binding site of TR contains charged and hydrophobic residues at its periphery, but only hydrophobic residues at its center (see, e.g., Figures 5 and 18). The hydrophobic cleft at the center of the site may play a significant role in driving the coactivator binding reaction. The site is comprised of two parts (Figure 18), right). Residues contained in helices 3, 5 and 6 (Figure 18, yellow residues) likely form a constitutive part, since their positions are identical in all nuclear receptor structures reported, including the liganded, activated states of the TR, RAR, and ER, the unliganded RXR, and the inhibitor-liganded ER. By contrast, the residues of helix 12 (Figure 18, red residues) are differently positioned in the active and inactive states reported. Thus the coactivator binding site for the nuclear receptors is likely to be formed in response to an active hormone by positioning helix 12 against a scaffold formed by helices 3-6. Because the coactivator binding site is so small, it is easy to understand how even slight changes in the position of helix 12, which may, for example, be induced by an antagonist ligand, could impair coactivator binding, and thus receptor activation.

B. ER coactivator binding site

Binding of the NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) to the ERα LBD buries 1000 Ų of predominantly hydrophobic surface area from both molecules. The NR-box 2 peptide binding site is a shallow groove composed of residues Leu 354, Val 355, Ile 358, Ala 361 and Lys 362 from helix 3; Phe 367 and Val 368 from helix 4; Leu 372 from the turn between helices 3 and 4; Gln 375, Val 376, Leu 379 and Glu 380 from helix 5; and Asp 538, Leu 539, Glu 542 and Met 543 from helix 12. The floor and sides of this groove are completely nonpolar, but the ends of this groove are charged. Therefore, structural characterization of the binding site of the NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) to the ERα LBD, which is the same NR-box 2 peptide utilized to crystallize the T₃-TR LBD, supports the findings for TR that residues forming the coactivator binding site of nuclear receptors is composed of a well defined hydrophobic cleft and a charged hydrophobic perimeter. These residues are highly conserved among the nuclear receptor super family (Figure 19). Structural characterization of the coactivator peptide-bound ER LBD also supports the concept of exploiting the slight differences among the coactivator binding sites of nuclear receptors in designing and identifying compounds that target specific nuclear receptors.

The ERα LBD interacts primarily with the hydrophobic face of the NR-box 2 peptide 686-KHKILHRLLQDSS-698 (residues 12-24 of SEQ ID NO: 6) α helix formed by the side chains of Ile 689 and the three (SEQ ID NO: 1) LxxLL motif leucines (Leu 690, Leu 693 and Leu 694). The side chain of Leu 690 is deeply embedded within the groove and forms van der Waals contacts with the side chains of Ile 358, Val 376, Leu 379, Glu 380 and Met 543. The side chain of Leu 694 is similarly isolated within the groove and makes van der Waals contacts with the side chains of Ile 358, Lys 362, Leu 372, Gln 375, Val 376 and Leu 379. In contrast, the side chains of both Ile 689 and the second NR box leucine, Leu 693, rest against the rim of the groove. The side chain of Ile 689 lies in a shallow depression formed by the side chains of Asp 538, Leu 539 and Glu 542. The side chain of Leu 693 makes nonpolar contacts with the side chains of Ile 358 and Leu 539.

The charged and polar side chains which form the hydrophilic face of the peptide helix project away from the ERα receptor and either interact predominantly with solvent or form symmetry contacts. None of the side chains of the polar and charged residues outside the helical region of either peptide in the asymmetric unit, with the exception of Lys 688 of peptide B, is involved in hydrogen bonds or salt bridges with its associated ERα LBD monomer. The ε-amino group of Lys 688 of peptide B hydrogen bonds to the side chain carboxylate of Glu 380 of monomer B. This interaction is presumably a crystal artifact; the main chain atoms of the N-

terminal three residues of peptide B are displaced from monomer B and interact extensively with a symmetry-related ER α LBD.

In addition to interacting with the hydrophobic face of the peptide helix, the ERα LBD stabilizes the main chain conformation of the NR box peptide by forming capping interactions with both ends of the peptide helix. Glu 542 and Lys 362 are positioned at opposite ends of the peptide binding site. The side chains of Glu 542 and Lys 362 form van der Waals contacts with main chain and side chain atoms at the N- and C-terminal turns of the peptide helix respectively. These interactions position the stabilizing charges of the γ-carboxylate of Glu 542 and ε-amino group of Lys 362 near the ends of the NR box peptide helix. The side chain carboxylate of Glu 542 hydrogen bonds to the amides of the residues of N-terminal turn of the peptide helix (residues 688 and 689 of peptide A; residues 689 and 690 of peptide B). Similarly, the ε-amino group of Lys 362 hydrogen bonds to the carbonyls of the residues of the C-terminal turn of the peptide helix (residue 693 of peptide A; residues 693 and 694 of peptide B).

Except for the orientation of helix 12, the structure of the peptide binding groove of the ER α LBD is almost identical in the DES and OHT complexes. The region of this groove outside of helix 12 is referred to herein as the "static region" of the NR box binding site. Helix 12 in the OHT complex and the NR box peptide helix in the DES complex interact with the static region of the coactivator recognition groove in strikingly similar ways.

Helix 12 mimics the hydrophobic interactions of the NR box peptide with the static region of the groove with a stretch of residues (residues 540 to 544) that resembles an NR box ((residues 6-10 of SEQ ID NO: 43) <u>LLEML</u> instead of (SEQ ID NO: 1) LxxLL). The side chains of Leu 540 and Met 543 lie in approximately the same locations as those of the first and second motif leucines (Leu 690 and Leu 693) in the peptide complex. Leu 540 is inserted into the groove and makes van der Waals contacts with Leu 354, Val 376 and Glu 380. Met 543 lies along the edge of the groove and forms van der Waals contacts with the side chains of Leu 354, Val 355 and Ile 358. The side chain position of Leu 544 almost exactly overlaps that of the third NR box leucine, Leu 694. Deep within the groove, the Leu 544 side chain makes van der Waals contacts with the side chains of Ile 358, Lys 362, Leu 372, Gln 375, Val 376 and Leu 379.

Helix 12 in the OHT complex is also stabilized by N- and C-terminal capping interactions. Lys 362 interacts with the C-terminal turn of helix 12 much as it does with the equivalent turn of the peptide helix. The Lys 362 side chain packs against the C-terminal turn of the helix 12 with its ε-amino group hydrogen bonding to the carbonyls of residues 543 and 544. Given that the capping

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interaction at the N-terminal turn coactivator helix is formed by a helix 12 residue (Glu 542), the N-terminal turn of helix 12 in the antagonist complex is forced to interact with another residue, Glu 380. The Glu 380 γ-carboxylate forms van der Waals contacts with Tyr 537 and interacts with the amide of Tyr 537 through a series of water-mediated hydrogen bonds.

In addition to forming these "NR box-like" interactions, helix 12 also forms van der Waals contacts with areas of the ERα LBD outside of the coactivator recognition groove. The side chain of Leu 536 forms van der Waals contacts with Glu 380 and Trp 383 and that of Tyr 537 forms van der Waals contacts with His 373, Val 376 and Glu 380. As a result of these contacts, helix 12 in the OHT complex buries more solvent accessible surface area (~1200 Ų) than the NR box peptide in the DES-ERα LBD-peptide complex.

Identification and characterization of the coactivator binding site for TR, and extension of this information to other nuclear receptors shows that this site is common for all nuclear receptors identified to date. Additionally, sequence and structural comparison, coupled with the Examples showing differential specificity for coactivator binding to TR, GR and ER, reveal that minor differences between the receptors, such as found in helix 12, are likely to influence specificity of a coactivator for different types of nuclear receptors. Thus, the Examples presented herein demonstrate that information derived from the structure and function of the TR coactivator binding site can be applied in design and selection of compounds that modulate binding of coactivator proteins to nuclear receptors for all members of the nuclear receptor super family.

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All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A method of identifying a compound that modulates coactivator binding to a nuclear receptor, said method comprising:

modeling test compounds that fit spacially into a nuclear receptor coactivator binding site of interest using an atomic structural model of a nuclear receptor coactivator binding site or portion thereof,

screening said test compounds in an assay characterized by binding of a test compound to a nuclear receptor coactivator binding site, and

identifying a test compound that modulates coactivator binding to said nuclear receptor.

- 2. The method of claim 1, wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues of human thyroid receptor selected from the group consisting of Val284, Phe293, Ile302, Leu305, and Leu454.
- 3. The method of claim 1, wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues of human thyroid receptor selected from the group consisting of Val284, Lys288, Ile302, Lys306, Leu454 and Glu457.
- 4. The method of claim 1, wherein said atomic structural model comprises atomic coordinates of amino acid residues corresponding to residues of human thyroid receptor helix 3 residues Ile280, Thr281, Val283, Val284, Ala287, and Lys288, helix 4 residue Phe293, helix 5 residues Gln301, Ile302, Leu305, Lys306, helix 6 residue Cys309, and helix 12 residues Pro453, Leu454, Glu457, Val458 and Phe459.
- 5. The method of claim 1, wherein said nuclear receptor coactivator binding site comprises amino acid residues corresponding to residues of human thyroid receptor selected from the group consisting of helix 3 residues Ile280, Thr281, Val283, Val284, Ala287, and Lys288, helix 4 residue Phe293, helix 5 residues Gln301, Ile302, Leu305, Lys306, helix 6 residue Cys309, and helix 12 residues Pro453, Leu454, Glu457, Val458 and Phe459.
- 6. The method of claim 5, wherein said amino acid residues corresponding to residues of human thyroid receptor comprise Val284, Phe293, Ile302, Leu305, and Leu454.

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- 7. The method of claim 5, wherein said amino acid residues corresponding to residues of human thyroid receptor comprise Val284, Lys288, Ile302, Lys306, Leu454 and Glu457.
- 8. The method of claim 1, wherein said nuclear receptor coactivator binding site comprises amino acid residues corresponding to residues of human thyroid receptor of helix 3 residues Ile280, Thr281, Val283, Val284, Ala287, and Lys288, helix 4 residue Phe293, helix 5 residues Gln301, Ile302, Leu305, Lys306, helix 6 residue Cys309, and helix 12 residues Pro453, Leu454, Glu457, Val458 and Phe459.
- 15 9. The method of any one of claims 5 through 8, wherein said nuclear receptor is selected from the group consisting of TR, RAR, RXR, PPAR, VDR, ER, GR, PR, MR, and AR.
 - 10. The method of claim 1, wherein said screening is in vitro.
 - 11. The method of claim 10, wherein said screening is high throughput screening.
 - 12. The method of claim 1, wherein said assay is a biological assay.
 - 13. The method of claim 1, wherein said test compound is from a library of compounds.
 - 14. The method of claim 1, wherein said test compound is an agonist or antagonist of coactivator binding.
- 15. The method of claim 14, wherein said test compound is a small organic molecule, a peptide, or peptidomimetic.
 - 16. The method of claim 15, wherein said compound is a peptide comprising a NR-box amino acid sequence, or derivative thereof.
- 35 17. A method for identifying an agonist or antagonist of coactivator binding to a nuclear receptor, said method comprising the steps of:

providing the atomic coordinates of a nuclear receptor coactivator binding site or portion thereof to a computerized modeling system;

modeling compounds which fit spacially into the nuclear receptor coactivator binding site; and

identifying in an assay for nuclear receptor activity a compound that increases or decreases the activity of said nuclear receptor by binding the coactivator binding site of said nuclear receptor, whereby an agonist or antagonist of coactivator binding is identified.

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- 18. A machine-readable data storage medium, comprising a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using said data, is capable of displaying a graphical three-dimensional representation of a molecular complex of a compound bound to a nuclear receptor coactivator binding site comprising structure coordinates of amino acids corresponding to human thyroid receptor amino acids selected from the group consisting of helix 3 residues Ile280, Thr281, Val283, Val284, Ala287, and Lys288, helix 4 residue Phe293, helix 5 residues Gln301, Ile302, Leu305, Lys306, helix 6 residue Cys309, and helix 12 residues Pro453, Leu454, Glu457, Val458 and Phe459, or a homologue of said molecular complex, wherein said homologue comprises a coactivator binding site that has a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5Å.
- 19. The machine readable storage medium of claim 18, wherein said nuclear receptor is a thyroid receptor.
- 20. The machine readable storage medium of claim 19, wherein said thyroid receptor is human.
 - 21. The machine readable storage medium of claim 20, wherein said molecule is peptide.
- 30 22. The machine readable storage medium of claim 21, wherein said peptide comprises a NR-box amino acid sequence, or derivative thereof.
 - 23. The machine-readable data storage medium according to claim 18, wherein said molecular complex is defined by the set of structure coordinates depicted in Appendix 1, or a homologue of said molecular complex, said homologue having a root mean square deviation from the backbone atoms of said amino acids of not more than 1.5Å.
 - 24. A machine-readable data storage medium comprising a data storage material encoded with a first set of machine readable data which, when combined with a second set of

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- machine readable data, using a machine programmed with instructions for using said first set of data and said second set of data, can determine at least a portion of the structure coordinates corresponding to the second set of machine readable data, wherein: said first set of data comprises a Fourier transform of at least a portion of the structural coordinates selected from the group consisting of coordinates depicted in Appendix 1; and said second set of data comprises an X-ray diffraction pattern of a molecule or molecular complex.
 - 25. A cocrystal of a nuclear receptor comprising a molecule bound to the coactivator binding site of said nuclear receptor.
- 15 26. The cocrystal of claim 25, wherein said nuclear receptor is a thyroid receptor.
 - 27. The cocrystal of claim 26, wherein said thyroid receptor is human.
 - 28. The cocrystal of claim 27, wherein said molecule is peptide.
 - 29. The cocrystal of claim 28, wherein said peptide comprises a NR-box amino acid sequence or derivative thereof.
 - 30. A compound identified according to the method of claim 1.

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Appendix 1

Atomic Coordinates for Human TR-B Complexed With T3, and a GRIP1 NR-box 2 Peptide

```
REMARK full length numbering
    REMARK all residue names correct
10
    REMARK peptide sequence
    REMARK two molecules of TRB - CHAIN A and CHAIN B
    REMARK two molecules of T3 - CHAIN J and CHAIN K
    REMARK two molecules of GRIP-1 peptide - CHAIN X and CHAIN Y
    REMARK chain X lies between A and B
15
    REMARK chain Y interacts with B only
    REMARK residues differing between A and B include:
    REMARK A 217 Glu, A 252 Gln, A 263 Lys (missing side chains)
    REMARK B 237 Ser, B239 His, B 394 Lys (missing side chains)
    REMARK additionally Gly 261, Gly 262 are not visible in chain A
20
    REMARK residues differing between X and Y include:
    REMARK A 692 Arg
    REMARK additionally, residues Lys 688, Lys 689; Ser 697, Ser 698
    REMARK are not visible in chain Y
                                                         35.239
                                                                 1.00 45.76
25
                       LYS A 211
                                       52.546
                                                23.912
    MOTA
               1
                   Ν
                                                                 1.00 43.42
                                       52.944
                                                24.345
                                                         36.586
                                                                                6
               2
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                       LYS A 211
    MOTA
                                                                 1.00 35.68
                                                                                6
                                                23.665
                                                         37.836
                       LYS A 211
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                   C
    MOTA
               3
                                                         37.763
                                                                 1.00 33.58
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                4
                   0
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                                                                                6
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                                                25.825
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		ATOM	296	0	ASP Z			22.775	36.021	50.113	1.00	46.02	8
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	25	ATOM	309	CG2	ILE .	Α	302	17.818	37.788	48.342	1.00	31.98	6
		ATOM	310	CG1	ILE .	Α	302	17.502	35.673	49.581	1.00	40.77	6
111		ATOM	311	CD1	ILE .			17.003	34.897	48.385	1.00	45.43	6
		ATOM	312	C	ILE .			20.698	37.268	48.096	1.00	38.58	6
		ATOM	313	0	ILE .			20.960	37.453	46.906		40.81	8
	30	ATOM	314	N	ILE .			21.228	37.972	49.097		37.50	7
·	30		315	CA	ILE .			22.179	39.060	48.874		39.33	6
81		ATOM						23.023	39.338	50.109		39.06	6
		ATOM	316	CB	ILE .			23.023	40.522	49.861		36.19	6
IJ.		ATOM	317	CG2	ILE.					51.313		40.15	6
iii	2.5	ATOM	318	CG1	ILE .			22.141	39.653				6
	35	ATOM	319	CD1	ILE			22.916	39.806	52.589		36.93	
111		MOTA	320	С	ILE			23.093	38.705	47.722		36.49	6
iii		MOTA	321	0	ILE			23.354	39.509	46.835		36.58	8
, 132		MOTA	322	N	LEU			23.580	37.477	47.762		32.91	7
		MOTA	323	CA	LEU			24.465	36.964	46.734		27.55	6
	40	MOTA	324	СВ	LEU	Α	304	24.935	35.554	47.123		22.35	6
		MOTA	325	CG	LEU	Α	304	26.150	35.480	48.029		26.88	6
		MOTA	326	CD1	LEU	Α	304	26.267	36.731	48.876		24.82	6
		MOTA	327	CD2	LEU	Α	304	26.084	34.226	48.861	1.00	23.69	6
		ATOM	328	С	LEU	Α	304	23.764	36.968	45.389	1.00	28.05	6
	45	MOTA	329	0	LEU	Α	304	24.212	37.623	44.443	1.00	24.68	8
		MOTA	330	N	LEU			22.657	36.236	45.318	1.00	26.34	7
		ATOM	331	CA	LEU			21.892	36.147	44.089	1.00	30.91	6
		ATOM	332	СВ	LEU			20.565	35.434	44.359	1.00	32.50	6
		ATOM	333	CG	LEU			20.637	33.950	44.635		33.36	6
	50	ATOM	334		LEU			19.247	33.370	44.779		33.87	6
	50		335		LEU			21.340	33.280	43.466		31.72	6
		ATOM			LEU			21.665	37.524	43.477		29.76	6
		ATOM	336	C						42.301		29.33	8
		MOTA	337	0	LEU			21.954	37.747				
		ATOM	338	N	LYS			21.157	38.439	44.298		29.72	7
	55	ATOM	339	CA	LYS			20.868	39.800	43.864		34.28	6
		ATOM	340	CB	LYS			20.293	40.615	45.026		35.98	6
		MOTA	341	CG	LYS			18.919	40.163	45.511		43.35	6
		ATOM	342	CD	LYS	A	306	18.397	41.127	46.559	1.00	51.50	6

	5	ATOM	775	CG	LEU A	Δ.	365	36.990	31.150	29.185	1 00	38.91	6
	3	ATOM	776		LEU A				32.216	30.036		34.47	6
			777		LEU A				30.883	29.663		34.24	6
		ATOM										26.23	6
		ATOM	778	C	LEU A				27.590	28.262			
		MOTA	779	0	LEU A				27.649	27.472		27.06	8
	10	MOTA	780	N	ASP A				26.528	29.021		25.23	7
		ATOM	781	CA	ASP A				25.358	28.988	1.00		6
		MOTA	782	CB	ASP A	. F	366	36.027	24.049	29.033		29.68	6
		MOTA	783	CG	ASP A	A .	366	36.799	23.874	30.303	1.00	35.74	6
		ATOM	784	OD1	ASP A	Α.	366	36.285	24.177	31.402	1.00	36.78	8
	15	ATOM	785	OD2	ASP A	. E	366	37.959	23.386	30.240	1.00	41.23	8
		ATOM	786	С	ASP A	. A	366	34.278	25.434	30.181	1.00	27.70	6
		ATOM	787	0	ASP A	. A	366	34.587	26.097	31.173	1.00	31.94	8
		ATOM	788	N	ASP A			33.141	24.743	30.066	1.00	29.18	7
		ATOM	789	CA	ASP A			32.120	24.679	31.120	1.00	32.72	6
	20	ATOM	790	СВ	ASP Z			31.472	23.284	31.147		38.04	6
	20	ATOM	791	CG	ASP A			30.806	22.924	29.854		42.43	6
		ATOM	792		ASP Z			29.877	23.650	29.409		35.95	8
		ATOM	793		ASP A			31.186	21.884	29.250		51.42	8
			794	C	ASP A			32.754	24.969	32.482		33.71	6
	25	ATOM			ASP A			32.484	26.000	33.098		38.30	8
151	25	ATOM	795	O				33.602	24.032	32.919		31.06	7
		ATOM	796	N	THR A							26.28	6
		ATOM	797	CA	THR A			34.329	24.124	34.181			
		ATOM	798	СВ	THR A			35.559	23.222	34.141		27.30	6
≒. i .	• •	ATOM	799	OG1	THR A			35.161	21.871	33.885		33.42	8
l#	30	ATOM	800	CG2	THR A			36.323	23.303	35.454		25.16	6
		ATOM	801	С	THR A			34.764	25.557	34.479		21.13	6
3: 2=20)		ATOM	802	0	THR Z			34.408	26.153	35.503		23.17	8
		ATOM	803	N	GLU A			35.545	26.092	33.551		21.32	7
1,20		ATOM	804	CA	GLU Z			36.065	27.435	33.661		28.00	6
	35	ATOM	805	СВ	GLU Z			36.960	27.707	32.453		32.79	6
ind M		MOTA	806	CG	GLU A			38.089	26.663	32.346		36.29	6
1		MOTA	807	CD	GLU A	A	369	38.906	26.747	31.110		41.03	6
1,1,2		ATOM	808	OE1	GLU A	Ą	369	38.337	26.744	29.994		42.05	8
		ATOM	809	OE2	GLU Z	A	369	40.158	26.795	31.218	1.00	42.03	8
	40	ATOM	810	С	GLU Z	A	369	34.953	28.471	33.821	1.00	25.57	6
		ATOM	811	0	GLU Z	A	369	34.987	29.256	34.760	1.00	20.56	8
		ATOM	812	N	VAL Z	Α	370	33.967	28.463	32.921	1.00	25.39	7
		ATOM	813	CA	VAL Z	A	370	32.849	29.396	33.029	1.00	25.99	6
		ATOM	814	СВ	VAL 2	Α	370	31.763	29.131	31.987	1.00	26.15	6
	45	ATOM	815		VAL 2			30.609	30.093	32.183	1.00	27.65	6
		ATOM	816		VAL 2			32.306	29.251	30.592	1.00	17.70	6
		ATOM	817	С	VAL			32.245	29.209	34.412		26.49	6
		ATOM	818	0	VAL 2			32.012	30.170	35.147		28.16	8
		ATOM	819	N	ALA			31.988	27.947	34.739		21.01	7
	50	ATOM	820	CA	ALA			31.393	27.554	36.011		19.57	6
	30				ALA			31.441	26.039	36.145		18.62	6
		ATOM	821	CB				32.116	28.211	37.177		23.48	6
		ATOM	822	C	ALA				28.989	37.931		32.67	8
		ATOM	823	0	ALA			31.531					7
	ہے ہے	ATOM	824	N	LEU .			33.401	27.893	37.305		22.89	
	55	ATOM	825	CA	LEU .			34.217	28.447	38.369		23.28	6
		ATOM	826	СВ	LEU			35.675	27.996	38.178		27.76	6
		ATOM	827	CG	LEU .			35.943	26.524	38.415		21.18	6
		MOTA	828	CD1	LEU	A	3/2	37.356	26.171	38.049	1.00	27.64	6

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	5	7 m () v	1477	CD1	PHE	7\	151	9.426	47.507	33.963	1.00	62.92	6
	3	ATOM			PHE			11.089	47.017	35.593		63.07	6
		ATOM	1478					10.199	48.598	33.521	1.00		6
		MOTA	1479		PHE						1.00		6
		ATOM	1480		PHE			11.860	48.102	35.156			6
		MOTA	1481	CZ	PHE			11.410	48.897	34.118		67.12	
	10	MOTA	1482	С	PHE			8.259	43.264	35.260		56.41	6
		ATOM	1483	0	PHE			7.641	43.392	36.331		56.56	8
		MOTA	1484	N	PRO			8.555	42.045	34.755		53.28	7
		ATOM	1485	CD	PRO			9.177	41.689	33.481		50.46	6
		ATOM	1486	CA	PRO	Α	452	8.153	40.859	35.543		50.26	6
	15	ATOM	1487	CB	PRO	А	452	8.739	39.680	34.780		49.19	6
		ATOM	1488	CG	PRO	Α	452	9.178	40.206	33.482		45.89	6
		MOTA	1489	С	PRO	Α	452	8.770	40.999	36.935		49.62	6
		ATOM	1490	0	PRO	Α	452	9.867	41.529	37.094		52.35	8
		MOTA	1491	N	PRO	Α	453	8.139	40.425	37.947		51.50	7
	20	ATOM	1492	CD	PRO	Α	453	7.001	39.542	37.797	1.00	49.66	6
		MOTA	1493	CA	PRO	Α	453	8.610	40.528	39.323	1.00	50.89	6
		ATOM	1494	СВ	PRO	Α	453	7.675	39.659	40.109	1.00	51.49	6
		ATOM	1495	CG	PRO	Α	453	6.703	39.141	39.185	1.00	50.82	6
		ATOM	1496	С	PRO	Α	453	10.015	40.084	39.532	1.00	50.99	6
	25	ATOM	1497	0	PRO			10.876	40.900	39.838	1.00	54.17	8
		MOTA	1498	N	LEU			10.255	38.781	39.423	1.00	51.21	7
iğ Li		ATOM	1499	CA	LEU			11.585	38.298	39.674	1.00	47.17	6
		ATOM	1500	СВ	LEU			11.813	36.962	38.975	1.00	44.44	6
, tall		ATOM	1501	CG	LEU			13.167	36.375	39.289	1.00	41.33	6
i.i	30	ATOM	1502	CD1				13.524	36.638	40.720	1.00	35.93	6
	50	ATOM	1503	CD2	LEU			13.169	34.907	38.992	1.00	34.79	6
#		ATOM	1504	С			454	12.541	39.375	39.182	1.00	42.25	6
		ATOM	1505	0	LEU			13.477	39.718	39.886	1.00	40.82	8
W		ATOM	1506	N			455	12.270	39.957	38.011	1.00	39.29	7
1.J	35	ATOM	1507	CA			455	13.133	41.005	37.473	1.00	41.81	6
	55	ATOM	1508	СВ			455	12.527	41.592	36.192	1.00	47.22	6
		ATOM	1509	CG			455	13.433	42.565	35.467	1.00	56.97	6
111		ATOM	1510	CD1	PHE			14.715	42.189	35.135	1.00	57.23	6
		ATOM	1511		PHE			12.999	43.840	35.126	1.00	59.40	6
	40	ATOM	1512		PHE			15.557	43.059	34.466	1.00	56.58	6
	10	ATOM	1513		PHE			13.848	44.716	34.452	1.00	61.80	6
		MOTA	1514	CZ			455	15.129	44.322	34.126		59.94	6
		ATOM	1515	C			455	13.273	42.085	38.534		45.12	6
		ATOM	1516	0			455	14.361	42.323	39.034		39.95	8
	45	ATOM	1517	N			456	12.155	42.735	38.849		43.92	7
	73	ATOM	1518	CA			456	12.122	43.803	39.840		44.08	6
		ATOM	1519	CB			456	10.680	44.251	40.093		50.20	6
		ATOM	1520	CG			456	10.062	45.242	39.144		55.79	6
			1521		LEU			8.598	45.432	39.450		54.70	6
	50	ATOM	1521		LEU			10.807	46.548	39.295		53.01	6
	50	ATOM	1523	CDZ			456	12.739	43.355	41.136		44.65	6
		ATOM					456	13.597	44.022	41.685		45.93	8
		ATOM	1524	0				11.973	41.761	41.851		44.56	7
		ATOM	1525	N			457	12.475	41.701	43.105		46.37	6
		ATOM	1526	CA			457		41.179	43.103		43.60	6
	55	ATOM	1527	С			457	14.005 14.583	41.236	43.132		42.69	8
		ATOM	1528	0			457		39.723	43.223		50.16	6
		ATOM	1529	CB			457 457	12.024 11.114	39.723	43.223		20.00	6
		ATOM	1530	CG	GПО	А	407	TT.TT4	33.410	33.34 <i>1</i>	1.00	20.00	J

	5	ATOM	1531	CD	GLU	Α	457	10.807	37.994	44.648		20.00	6
		MOTA	1532	OE1	GLU	Α	457	11.673	37.099	44.311		20.00	8
		MOTA	1533	OE2	GLU	Α	457	9.683	37.639	45.172	1.00	20.00	8
		ATOM	1534	N	VAL	Α	458	14.928	41.078	41.903	1.00	43.21	7
		ATOM	1535	CA	VAL	Α	458	16.412	41.094	41.868	1.00	44.98	6
	10	ATOM	1536	СВ	VAL	Α	458	16.881	40.306	40.642	1.00	44.83	6
		MOTA	1537	CG1	VAL	Α	458	18.365	40.106	40.698	1.00	49.72	6
		ATOM	1538		VAL			16.185	38.979	40.558	1.00	40.89	6
		ATOM	1539	С	VAL			17.130	42.420	41.877	1.00	42.72	6
		MOTA	1540	0	VAL			18.061	42.617	42.658	1.00	42.88	8
	15	ATOM	1541	N	PHE			16.713	43.325	41.010	1.00	44.53	7
		ATOM	1542	CA	PHE			17.385	44.606	40.892	1.00	48.18	6
		ATOM	1543	СВ	PHE			17.281	45.104	39.494	1.00	43.60	6
		MOTA	1544	CG	PHE			17.915	44.190	38.547	1.00	40.79	6
		ATOM	1545		PHE			17.325	42.983	38.244		41.01	6
	20	ATOM	1546		PHE			19.153	44.483	38.054		39.48	6
	20	ATOM	1547	CE1	PHE			17.988	42.081	37.441		40.62	6
		ATOM	1548	CE2	PHE			19.814	43.589	37.257	1.00	36.87	6
		ATOM	1549	CZ	PHE			19.233	42.385	36.940	1.00	36.39	6
inci		ATOM	1550	C	PHE			16.837	45.648	41.744	1.00		6
	25		1551	0			459	17.492	46.682	42.017		51.34	8
N	23	ATOM	1552	N	GLU			15.606	45.422	42.161		62.92	7
H		MOTA	1553	CA			460	15.066	46.428	42.965		69.33	6
i.e.s		ATOM	1553	CB			460	13.552	46.352	43.094		72.95	6
		ATOM					460	12.978	47.767	42.957		78.35	6
	20	MOTA	1555	CG			460	12.246	48.261	44.157		82.97	6
1	30	ATOM	1556	CD				12.471	47.759	45.281		88.28	8
==		ATOM	1557	OE1	GLU			11.422	49.200	44.017		84.80	8
		ATOM	1558	OE2	GLU			15.736	46.245	44.272		71.87	6
		ATOM	1559	С			460	16.187	45.170	44.691		74.51	8
IJ	25	ATOM	1560	0			460	15.790	47.373	44.917		78.50	7
	35	ATOM	1561	N	ASP			16.415	47.505	46.173		84.19	6
		ATOM	1562	CA			461	16.394	48.981	46.471		85.82	6
ıİ.		ATOM	1563	CB			461		49.786	45.276		89.62	6
		ATOM	1564	CG			461	16.801 16.692	49.766	44.086		93.00	8
	40	ATOM	1565		ASP			17.239	50.923	45.482		93.04	8
	40	ATOM	1566		ASP			15.639	46.703	47.214		86.80	6
		ATOM	1567	C			461	16.245	45.748	47.731		88.70	8
		ATOM	1568	0			461		47.026	47.451		88.70	8
		ATOM	1569	OXT	ASP	А	461	14.457	47.020	47.401	1.00	00.70	O
	4.5	TER	-1	O.D.	T 170	-	011	20 002	66 251	39.780	1 00	46.72	6
	45	ATOM	1	CB			211	-20.802	66.251	39.760		56.48	6
		MOTA	2	CG			211	-19.566	65.345			60.93	6
		MOTA	3	CD			211	-18.264	66.114	40.045		61.95	6
		MOTA	4	CE			211	-18.043	67.067	38.886		69.93	7
		ATOM	5	NΖ			211	-19.008	68.224	38.903		35.68	6
	50	ATOM	6	С			211	-22.418	67.861	40.818		33.58	8
		ATOM	7	0			211	-23.356	67.113	40.454			
		MOTA	8	N			211	-20.742	66.675	42.239		45.76	7
		MOTA	9	CA			211	-20.998	67.285	40.894		43.42	6
		MOTA	10	N			212	-22.610	69.205	41.068		35.64	7
	55	MOTA	11	CD			212	-21.526	70.177	41.287		38.60	6
		ATOM	12	CA			212	-23.943	69.861	41.036		38.35	6
		MOTA	13	СВ			212	-23.657	71.320	41.420		38.95	6
		MOTA	14	CG	PRO	В	212	-22.226	71.474	41.551	1.00	42.00	6

	5	7 TCM	15	С	PRO	B	212	-24.798	69.772	39.807	1.00	38.78	6
	5	ATOM	16	0	PRO			-24.350	70.045	38.696		34.64	8
		MOTA			GLU			-24.330	69.424	40.032		40.31	7
		MOTA	17	N	GLU			-27.081	69.290	39.003		43.87	6
		ATOM	18	CA	GLU			-27.895	68.004	39.265		45.16	6
	10	ATOM	19	CB				-27.033	66.709	39.286		47.60	6
	10	ATOM	20	CG	GLU			-27.032 -27.807	65.421	39.199		50.68	6
		ATOM	21	CD	GLU				65.244	39.886		59.18	8
		MOTA	22		GLU			-28.847	64.516	38.442		49.06	8
		ATOM	23		GLU			-27.382	70.576	39.080		45.96	6
	1.5	ATOM	24	С	GLU			-27.924	71.467			43.13	8
	15	ATOM	25	0	GLU			-27.624		39.859 38.308		46.52	7
		MOTA	26	N	PRO			-28.987	70.698			46.44	6
		ATOM	27	CD	PRO		214	-29.484	69.635	37.446		47.52	6
		ATOM	28	CA	PRO		214	-29.843	71.907	38.302			6
	• •	MOTA	29	СВ	PRO			-30.799	71.639	37.210		45.40	6
	20	ATOM	30	CG	PRO			-30.530	70.257	36.805		49.89 45.70	6
		ATOM	31	С	PRO			-30.574	72.330	39.535			
		ATOM	32	0	PRO			-30.597	71.595	40.483		44.49	8 7
		ATOM	33	N	THR			-31.180	73.515	39.506		45.24	
i mar		ATOM	34	CA			215	-31.965	74.036	40.652		49.36	6
1	25	ATOM	35	CB			215	-31.443	75.420	41.091		44.86	6
ij		ATOM	36	OG1	THR			-32.249	76.464	40.534		52.26	8
IJ		ATOM	37	CG2			215	-30.011	75.617	40.659		39.43	6
		MOTA	38	С			215	-33.386	74.239	40.114		52.51	6
		ATOM	39	0			215	-33.562	74.868	39.078		53.48	8
i Li	30	ATOM	40	N			216	-34.387	73.741	40.829		58.81	7
		MOTA	41	CA			216	-35.795	73.865	40.435		61.51	6
####		ATOM	42	СВ			216	-36.674	74.005	41.650		70.57	6
i		MOTA	43	CG			216	-37.675	72.981	41.710	1.00	78.07	6
1:4		ATOM	44		ASP			-38.228	72.588	40.652		82.31	8
	35	ATOM	45		ASP			-37.983	72.567	42.830		86.55	8
		ATOM	46	С			216	-35.920	75.123	39.648		58.42	6
		ATOM	47	0			216	-36.847	75.317	38.827		56.85	8
*85		ATOM	48	N			217	-34.954	75.979	39.984		54.92	7
		ATOM	49	CA			217	-34.851	77.259	39.353		53.37	6
	40	MOTA	50	CB			217	-34.104	78.264	40.251		51.02	6
		ATOM	51	CG			217	-34.151	79.689	39.679		40.00	6
		ATOM	52	CD			217	-34.301	80.745	40.739		40.00	6
		MOTA	53		GLU			-34.089	80.443	41.945		40.00	8
		ATOM	54		GLU			-34.625	81.921	40.411		40.00	8
	45	MOTA	55	С			217	-34.232	77.163	37.957		53.55	6
		ATOM	56	0			217	-34.815	77.612	37.018		54.33	8
		MOTA	57	N			218	-33.063	76.572	37.839		49.20	7
		ATOM	58	CA			218	-32.318	76.385	36.608		45.94	6
		MOTA	59	CB			218	-30.965	75.793	36.981		43.43	6
	50	MOTA	60	CG			218	-30.065	76.728	37.801		40.86	6
		ATOM	61	CD			218	-28.713	76.159	38.072		39.88	6
		ATOM	62	OE1			218	-28.606	74.967	38.449		37.61	8
		MOTA	63	OE2			218	-27.707	76.901	37.945		34.01	8
		ATOM	64	С			218	-33.014	75.475	35.610		44.71	6
	55	MOTA	65	0			218	-32.935	75.686	34.405		45.31	8
		MOTA	66	N			219	-33.669	74.439	36.131		44.02	7
		ATOM	67	CA			219	-34.368	73.490	35.290		46.97	6
		MOTA	68	CB	TRP	В	219	-35.046	72.408	36.119	1.00	48.42	6

	5	7. 11.01.4	69	CG	TRP I	,	210	-34.195	71.230	36.374	1 00	54.61	6
	3	ATOM			TRP I		219	-34.048	70.120	35.478		55.24	6
		ATOM	70					-33.076	69.248	36.063		53.67	6
		ATOM	71		TRP I		219		69.771	34.252		54.55	6
		ATOM	72	CE3	TRP I		219	-34.615		37.415		55.75	6
	4.0	MOTA	73	CD1	TRP I		219	-33.399	71.019				7
	10	ATOM	74	NE1	TRP I		219	-32.697	69.838	37.236		54.43	
		ATOM	75	CZ2	TRP 1		219	-32.635	68.075	35.431		52.54	6
		ATOM	76	CZ3	TRP 1		219	-34.214	68.603	33.643		55.17	6
		ATOM	77	CH2			219	-33.234	67.758	34.214		55.59	6
		MOTA	78	С			219	-35.409	74.199	34.459	1.00	47.32	6
	15	MOTA	79	0			219	-35.561	73.914	33.277	1.00	43.56	8
		ATOM	80	N	GLU !		220	-36.126	75.130	35.084	1.00	49.91	7
		ATOM	81	CA	GLU I			-37.158	75.874	34.402		53.57	6
		MOTA	82	СВ	GLU I		220	-37.811	76.820	35.373	1.00	58.18	6
		MOTA	83	CG	GLU I	В 2	220	-39.251	76.812	35.221	1.00	73.13	6
	20	ATOM	84	CD	GLU I		220	-39.824	76.858	36.489		80.06	6
		ATOM	85	OE1	GLU :		220	-39.485	75.995	37.324		82.12	8
		ATOM	86	OE2	GLU :	В 2	220	-40.635	77.740	36.718	1.00	82.78	8
		ATOM	87	С	GLU :	В 2	220	-36.539	76.645	33.250	1.00	50.51	6
		ATOM	88	0	GLU :	В 2	220	-37.160	76.793	32.195	1.00	49.94	8
ı	25	ATOM	89	N	LEU :	в :	221	-35.312	77.135	33.455		43.71	7
		MOTA	90	CA	LEU :	в 2	221	-34.604	77.884	32.411	1.00	42.81	6
		ATOM	91	СВ	LEU :	в :	221	-33.214	78.324	32.865	1.00	39.21	6
j-£		ATOM	92	CG	LEU :	В	221	-32.321	78.833	31.754	1.00	36.34	6
الِه: "		ATOM	93	CD1	LEU	В	221	-33.073	79.843	30.927	1.00	36.93	6
	30	MOTA	94	CD2	LEU	В	221	-31.058	79.446	32.331	1.00		6
'aj		ATOM	95	С	LEU	В	221	-34.454	77.011	31.192	1.00		6
E:		ATOM	96	0	LEU	В	221	-34.819	77.406	30.104	1.00		8
		ATOM	97	N	ILE	В	222	-33.878	75.829	31.398	1.00	39.09	7
IJ.		ATOM	98	CA	ILE	В	222	-33.687	74.857	30.330	1.00	35.47	6
	35	MOTA	99	СВ	ILE	В	222	-33.224	73.516	30.871	1.00	33.74	6
		ATOM	100	CG2	ILE	В.	222	-33.204	72.488	29.776		28.86	6
1		ATOM	101	CG1	ILE	В	222	-31.840	73.631	31.493	1.00	33.33	6
144		ATOM	102	CD1	ILE	В	222	-31.435	72.419	32.264	1.00	34.85	6
		ATOM	103	С	ILE	В	222	-34.991	74.627	29.598		34.26	6
	40	ATOM	104	0	ILE	В	222	-35.082	74.832	28.392		31.90	8
		ATOM	105	N	LYS	В	223	-35.992	74.183	30.346	1.00	39.49	7
		ATOM	106	CA	LYS	В	223	-37.300	73.892	29.785	1.00	44.43	6
		ATOM	107	CB	LYS	В	223	-38.351	73.876	30.882		50.81	6
		ATOM	108	CG	LYS	В	223	-39.693	73.358	30.411		62.51	6
	45	ATOM	109	CD	LYS	В	223	-40.795	73.532	31.449		72.22	6
		ATOM	110	CE	LYS	В	223	-42.163	73.249	30.827	1.00	74.55	6
		ATOM	111	NZ	LYS	В	223	-43.268	73.378	31.837		75.78	7
		ATOM	112	С	LYS	В	223	-37.648	74.942	28.755		42.81	6
		ATOM	113	0	LYS	В	223	-38.337	74.661	27.796	1.00	40.36	8
	50	ATOM	114	N	THR	В	224	-37.146	76.156	28.979	1.00	39.89	7
		ATOM	115	CA	THR	В	224	-37.353	77.293	28.074	1.00	39.93	6
		MOTA	116	СВ	THR	В	224	-36.956	78.609	28.776	1.00	40.57	6
		MOTA	117	OG1	THR	В	224	-37.646	78.740	30.028	1.00	39.27	8
	55	ATOM	118		THR			-37.273	79.805	27.893		38.11	6
		ATOM	119	С	THR			-36.521	77.094	26.789	1.00	39.96	6
		MOTA	120	0	THR			-37.043	76.677	25.756		36.67	8
		ATOM	121	N	VAL			-35.231	77.421	26.888		38.02	7
		ATOM	122	CA	VAL			-34.263	77.295	25.801	1.00	38.12	6
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	5	ATOM	101	CA	LYS B	274	-31.983	79.774	-2.147	1.00 54.53	6
		ATOM	102	СВ	LYS B	274	-32.133	78.724	-3.232	1.00 54.36	6
		ATOM	103	С	LYS B	274	-32.819	79.396	-0.931	1.00 56.88	6
		ATOM	104	0	LYS B	274	-34.025	79.624	-0.906	1.00 57.98	8
		ATOM	105	N	ILE B		-32.151	78.820	0.076	1.00 56.48	7
	10	ATOM	106	CA	ILE B		-32.791	78.381	1.332	1.00 52.64	6
		ATOM	107	СВ	ILE B		-32.638	76.863	1.519	1.00 49.15	6
		ATOM	108	CG2	ILE B		-33.505	76.105	0.529	1.00 47.42	6
		ATOM	109	CG1	ILE B		-31.188	76.441	1.343	1.00 45.31	6
		ATOM	110	CD1	ILE B		-30.990	74.952	1.391	1.00 37.22	6
	15	ATOM	111	С	ILE B		-32.241	79.086	2.574	1.00 51.78	6
	10	ATOM	112	0	ILE B		-32.858	79.049	3.622	1.00 49.80	8
		ATOM	113	N	ILE B		-31.071	79.709	2.435	1.00 51.76	7
		ATOM	114	CA		276	-30.410	80.409	3.533	1.00 52.58	6
		ATOM	115	CB	ILE B		-29.145	81.110	3.042	1.00 55.04	6
	20	ATOM	116	CG2	ILE B		-29.486	82.172	2.017	1.00 53.28	6
	20	ATOM	117	CG1	ILE B		-28.396	81.786	4.203	1.00 57.31	6
		ATOM	118	CD1	ILE B		-27.862	80.854	5.231	1.00 60.32	6
		ATOM	119	C	ILE B		-31.282	81.461	4.237	1.00 50.70	6
		ATOM	120	0	ILE B		-31.015	81.817	5.385	1.00 55.55	8
i.	25	ATOM	121	N	THR B		-32.322	81.953	3.568	1.00 47.33	7
147	20	ATOM	122	CA	THR B		-33.174	82.968	4.141	1.00 42.59	6
		ATOM	123	СВ	THR B		-34.042	83.632	3.048	1.00 44.97	6
		ATOM	124	OG1	THR E		-33.202	84.145	2.001	1.00 46.38	8
1.1.0		ATOM	125	CG2	THR E		-34.856	84.781	3.653	1.00 37.17	6
1	30	ATOM	126	C	THR E		-34.069	82.447	5.267	1.00 39.84	6
	50	ATOM	127	0	THR E		-34.083	83.026	6.375	1.00 40.55	8
9.5		ATOM	128	N	PRO E		-34.832	81.385	5.017	1.00 38.20	7
		ATOM	129	CD	PRO E		-34.925	80.666	3.747	1.00 36.34	6
L		ATOM	130	CA	PRO E		-35.711	80.834	6.059	1.00 36.63	6
IJ	35	ATOM	131	СВ	PRO E		-36.475	79.715	5.357	1.00 32.95	6
	33	ATOM	132	CG	PRO E		-35.833	79.516	4.056	1.00 35.75	6
		ATOM	133	C	PRO E		-34.892	80.324	7.220	1.00 38.60	6
1		ATOM	134	0	PRO E		-35.372	80.157	8.331	1.00 37.67	8
		ATOM	135	N	ALA E		-33.636	80.040	6.927	1.00 37.05	7
	40	MOTA	136	CA	ALA E		-32.696	79.525	7.903	1.00 33.18	6
		MOTA	137	СВ	ALA E		-31.391	79.195	7.205	1.00 30.56	6
		ATOM	138	С	ALA E		-32.447	80.536	8.991	1.00 33.47	6
		ATOM	139	0	ALA E		-32.623	80.238	10.158	1.00 33.74	8
		ATOM	140	N	ILE E		-32.010	81.728	8.577	1.00 29.96	7
	45	ATOM	141	CA	ILE E		-31.728	82.809	9.501	1.00 25.94	6
		ATOM	142	СВ	ILE E		-31.190	84.040	8.754	1.00 26.95	6
		ATOM	143		ILE E		-30.881	85.149	9.715	1.00 15.40	6
		ATOM	144		ILE E		-29.904	83.696	8.007	1.00 26.73	6
		ATOM	145		ILE E		-29.255	84.878	7.362	1.00 34.31	6
	50	ATOM	146	С	ILE E		-32.964	83.172	10.310	1.00 31.39	6
	50	ATOM	147	0	ILE E		-32.882	83.378	11.522	1.00 35.69	8
		ATOM	148	N	THR E		-34.113	83.233	9.647	1.00 30.90	7
		ATOM	149	CA	THR E		-35.361	83.586	10.328	1.00 33.49	6
		ATOM	150	CB	THR I		-36.598	83.396	9.419	1.00 37.18	6
	55	ATOM	151	OG1			-36.703	82.034	9.005	1.00 46.48	8
	55	ATOM	152	CG2			-36.525	84.289	8.198	1.00 32.85	6
		ATOM	153	C		3 281	-35.523	82.706	11.556	1.00 29.94	6
		ATOM	154	0		3 281	-35.855	83.186	12.634	1.00 25.55	8
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	5	MOTA	641	С	GLY :	В	344	-19.867	77.636	-2.258		38.26	6
		MOTA	642	0	GLY :	В	344	-20.715	78.501	-2.484		35.69	8
		ATOM	643	N	GLY	В	345	-18.724	77.871	-1.619	1.00	35.89	7
		ATOM	644	CA	GLY	В	345	-18.426	79.209	-1.159	1.00	34.00	6
		ATOM	645	С	GLY	В	345	-17.848	79.298	0.230	1.00	38.64	6
	10	ATOM	646	0	GLY	В	345	-17.216	80.303	0.573	1.00	38.14	8
		MOTA	647	N	LEU	В	346	-18.071	78.266	1.041	1.00	39.52	7
		ATOM	648	CA	LEU			-17.563	78.279	2.403	1.00	36.05	6
		ATOM	649	СВ	LEU			-18.311	77.256	3.269	1.00	35.72	6
		ATOM	650	CG	LEU			-19.800	77.473	3.378	1.00	34.89	6
	15	ATOM	651	CD1	LEU		346	-20.322	76.678	4.554	1.00	44.09	6
	15	ATOM	652	CD2	LEU		346	-20.086	78.937	3.612	1.00	34.84	6
		ATOM	653	C	LEU		346	-16.079	78.018	2.445	1.00	33.52	6
		ATOM	654	0	LEU		346	-15.392	78.387	3.394		35.58	8
		ATOM	655	N	GLY		347	-15.586	77.388	1.385	1.00	30.47	7
	20	ATOM	656	CA	GLY		347	-14.174	77.078	1.305	1.00	33.01	6
	20	ATOM	657	C	GLY			-13.768	76.214	2.477	1.00	30.72	6
		ATOM	658	0	GLY		347	-14.433	75.243	2.808	1.00	30.89	8
		ATOM	659	N	VAL		348	-12.647	76.585	3.087		31.30	7
4=4		ATOM	660	CA	VAL		348	-12.097	75.867	4.227		31.27	6
ű	25	ATOM	661	CB	VAL		348	-10.889	76.609	4.817		31.66	6
N	23	ATOM	662	CG1	VAL		348	-11.292	77.974	5.360		20.19	6
ij		ATOM	663		VAL		348	-10.250	75.786	5.905		24.77	6
			664	C	VAL			-13.136	75.651	5.360		33.84	6
		ATOM	665	0	VAL			-13.002	74.707	6.153		29.99	8
	30	ATOM	666	N	VAL			-14.157	76.518	5.449		33.31	7
Ų	30	ATOM	667	CA	VAL		349	-15.147	76.339	6.483		32.23	6
H.		ATOM	668	CB	VAL		349	-16.226	77.393	6.476		32.59	6
		MOTA	669	CG1	VAL		349	-17.342	76.979	7.399		33.68	6
IJ		ATOM ATOM	670		VAL		349	-15.667	78.703	6.959		32.30	6
IJ	35		671	C	VAL		349	-15.792	74.987	6.380		34.91	6
	33	ATOM ATOM	672	0	VAL		349	-16.055	74.359	7.394		33.73	8
			673	N	SER		350	-16.054	74.507	5.176		32.81	7
ū		ATOM	674	CA	SER		350	-16.695	73.215	5.100		30.10	6
		ATOM ATOM	675	CB	SER		350	-16.772	72.697	3.684		24.95	6
	40	ATOM	676	OG	SER		350	-17.538	71.502	3.644		23.16	8
	40		677	C	SER			-15.910	72.254	5.942		31.59	6
		ATOM ATOM	678	0	SER			-16.417	71.807	6.950		37.62	8
		ATOM	679	N	ASP			-14.675	71.942	5.565		28.60	7
		ATOM	680	CA	ASP			-13.905	71.010	6.378		29.82	6
	45	ATOM	681	CB	ASP			-12.419	71.139	6.050		27.49	6
	43	ATOM	682	CG	ASP			-12.151	71.094	4.585		30.22	6
		ATOM	683		ASP			-12.013	72.174	3.954		32.61	8
		ATOM	684		ASP			-12.064	69.980	4.017		30.02	8
			685	C	ASP			-14.176	71.343	7.861		30.63	6
	50	ATOM			ASP			-14.458	70.474	8.681		29.54	8
	50	ATOM	686	O N			352	-14.111	72.629	8.177		25.33	7
		ATOM	687	N			352	-14.111	73.092	9.533		28.59	6
		ATOM	688	CA			352	-14.252	74.606	9.572		20.95	6
		ATOM	689	СВ				-14.232	72.630	10.086		29.69	6
	<i>55</i>	ATOM	690	С			352 352	-15.890 -15.757	72.030	11.164		30.36	8
	55	ATOM	691	O			352 353	-15.757 -16.754	72.000	9.330		27.63	7
		ATOM	692	N C7			353	-18.096	72.504	9.729		27.55	6
		ATOM	693	CA			353 353	-16.096 -19.144	73.129	8.800		28.04	6
		MOTA	694	CB	TTE	ם	333	-19.144	10.149	0.000	1.00	20.01	Ŭ

	5	ATOM	4035	C11	Т3	K	1	-26.708	75.670	7.834	1.00		6
		MOTA	4036	C12	Т3	K	1	-24.521	78.610	5.376		19.67	6
		ATOM	4037	C13	Т3	K	1	-29.211	75.830	8.626		18.97	6
		ATOM	4038	C15	Т3	K	1	-29.181	74.567	9.488		19.32	6
		MOTA	4039	C17	Т3	K	1	-30.440	74.343	10.264		19.02	6
	10	ATOM	4040	I1	Т3	K	1	-27.868	77.342	3.316		25.29	53
		ATOM	4041	12	Т3	K	1	-22.732	79.619	0.850		26.49	53
		ATOM	4042	13	Т3	K	1	-23.602	75.792	7.334		25.67	53
		ATOM	4043	N1	Т3	K	1	-28.680	73.342	8.762	1.00	15.12	7
		ATOM	4044	01	Т3	K	1	-22.742	81.265	3.443	1.00	21.79	8
	15	ATOM	4045	02	Т3	K	1	-25.267	76.388	4.595	1.00	22.05	8
		ATOM	4046	03	Т3	K	1	-30.816	73.159	10.382	1.00	20.38	8
		ATOM	4047	04	Т3	K	1	-31.028	75.359	10.729	1.00	20.16	8
		TER											
		ATOM	1	С	LYS	Х	686	13.868	40.176	48.888	1.00	40.00	6
	20	ATOM	2	0	LYS			13.914	40.120	47.639	1.00	40.00	8
	20	ATOM	3	N	LYS			14.374	42.245	50.489	1.00	40.00	7
		ATOM	4	CA	LYS			14.937	41.070	49.710	1.00	40.00	6
		ATOM	5	N	HIS			13.038	39.527	49.705	1.00	40.00	7
		ATOM	6	CA	HIS			11.891	38.518	49.521	1.00	40.00	6
	25	ATOM	7	CB	HIS			10.639	39.000	50.212	1.00	40.00	6
	23	ATOM	8	CG	HIS			10.981	39.526	51.563	1.00	40.00	6
1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		ATOM	9		HIS			11.021	38.908	52.753	1.00	40.00	6
		ATOM	10		HIS			11.354	40.844	51.754	1.00		7
		ATOM	11					11.614	40.994	53.034	1.00	40.00	6
] 20Es	30	ATOM	12		HIS			11.422	39.847	53.646		40.00	7
in the second	50	ATOM	13	C			687	11.183	38.108	48.208		40.00	6
##		ATOM	14	0			687	11.674	38.361	47.094	1.00	40.00	8
		ATOM	15	N			688	10.064	37.458	48.649	1.00	40.00	7
ijij		ATOM	16	CA			688	8.911	36.858	47.931	1.00	40.00	6
	35	ATOM	17	CB			688	8.292	37.850	46.968		40.00	6
	33	ATOM	18	C			688	9.246	35.573	47.161	1.00	40.00	6
		ATOM	19	0			688	9.319	34.473	47.722	1.00	40.00	8
111		ATOM	20	N			689	9.426	35.754	45.865	1.00	40.00	7
		ATOM	21	CA			689	9.661	34.640	44.924		40.00	6
	40	ATOM	22	СВ			689	9.731	35.167	43.498	1.00	40.00	6
	70	ATOM	23		ILE			9.638	34.053	42.453	1.00	40.00	6
		ATOM	24		ILE			8.597	36.141	43.176	1.00	40.00	6
		ATOM	25		ILE			8.250	36.183	41.688	1.00	40.00	6
		ATOM	26	C			689	10.954	33.869	45.228		40.00	6
	45	ATOM	27	0			689	10.920	32.657	45.511		40.00	8
	43	ATOM	28	N			690	12.065	34.579	45.140		40.00	7
		ATOM	29	CA			690	13.391	33.996	45.397		40.00	6
		ATOM	30	CB			690	14.349	35.043	45.892		40.00	6
		ATOM	31	CG			690	14.450	36.168	44.906		40.00	6
	50	ATOM	32				690	15.397	37.261	45.363		40.00	6
	50	ATOM	33				690	14.940	35.695	43.540		40.00	6
			34	CDZ			690	13.271	32.999	46.466		40.00	6
		ATOM					690	13.633	31.832	46.315		40.00	8
		MOTA	35 36	O N			691	12.773	33.472	47.541		40.00	7
	55	MOTA	36 37	N CA			691	12.773	32.559	48.569		40.00	6
	55	ATOM	38	CB			691	11.729	33.212	49.658		40.00	6
		ATOM	38 39	CB			691	12.588	34.116	50.564		40.00	6
		ATOM	39 40				691	13.648	33.852	51.385		40.00	6
		MOTA	40	CDZ	HTO	Λ	091	10.040	55.052	01.000			-

													_
	5	ATOM	95	0	SER	X	698	13.253	17.158	42.026		40.00	8
		MOTA	96	OXT	SER	Χ	698	13.131	18.976	40.714	1.00	40.00	8
		TER											
		ATOM	1	СВ	LYS	Y	688	-33.793	96.885	6.491	1.00	40.00	6
		ATOM	2	С	LYS	Y	688	-35.002	95.370	8.130	1.00	40.00	6
	10	ATOM	3	0	LYS			-36.027	95.520	8.779	1.00	40.00	8
	10	ATOM	4	N	LYS			-32.717	96.619	8.695	1.00	40.00	7
			5	CA	LYS			-34.040	96.591	7.954		40.00	6
		ATOM			ILE			-34.578	93.781	6.908		40.00	7
		ATOM	6	N				-35.862	93.106	7.268		40.00	6
		ATOM	7	CA	ILE					6.572		40.00	6
	15	ATOM	8	СВ	ILE			-35.971	91.759				6
		ATOM	9	CG2	ILE			-37.270	91.077	6.932		40.00	6
		ATOM	10	CG1	ILE			-35.917	91.937	5.062		40.00	
		ATOM	11	CD1	ILE			-36.341	90.691	4.289		40.00	6
		ATOM	12	С			689	-36.032	92.870	8.780		40.00	6
	20	MOTA	13	0	ILE	Y	689	-36.913	93.446	9.442		40.00	8
		ATOM	14	N	LEU	Υ	690	-35.019	92.834	9.787		40.00	7
		ATOM	15	CA	LEU	Y	690	-34.956	92.320	11.163		40.00	6
		ATOM	16	СВ	LEU	Y	690	-33.528	92.432	11.697	1.00	40.00	6
		MOTA	17	CG	LEU	Y	690	-32.516	91.647	10.864	1.00	40.00	6
i	25	ATOM	18		LEU			-31.087	91.764	11.397	1.00	40.00	6
n.	23	ATOM	19		LEU			-32.819	90.148	10.812	1.00	40.00	6
		ATOM	20	C	LEU			-35.899	93.123	12.065	1.00	40.00	6
ins jes		ATOM	21	0	LEU			-36.570	92.492	12.928		40.00	8
14							691	-36.039	94.731	11.373		40.00	7
ļā	20	ATOM	22	N			691	-36.634	94.923	12.683		40.00	6
٠	30	MOTA	23	CA					96.383	12.935		40.00	6
		ATOM	24	CB			691	-36.854		13.078		40.00	6
		ATOM	25	CG			691	-35.610	97.153			40.00	6
1 . I		MOTA	26	CD2				-34.757	97.640	12.159			7
IJ.		MOTA	27		HIS			-35.129	97.579	14.319		40.00	6
144 374	35	MOTA	28		HIS			-34.039	98.290	14.122		40.00	
		ATOM	29	NE2				-33.786	98.346	12.815		40.00	7
		ATOM	30	С			691	-37.972	94.287	12.756		40.00	6
1 L		ATOM	31	0	HIS	Y	691	-38.240	93.417	13.545		40.00	8
		ATOM	32	N	ARG	Y	692	-38.265	94.388	11.505		40.00	7
	40	ATOM	33	CA	ARG	Y	692	-39.577	93.869	11.276	1.00	40.00	6
		ATOM	34	СВ	ARG	Y	692	-39.653	93.692	9.795		40.00	6
		ATOM	35	CG	ARG	Y	692	-40.759	92.764	9.329	1.00	40.00	6
		ATOM	36	CD			692	-40.618	92.422	7.848	1.00	40.00	6
		ATOM	37	NE			692	-41.849	92.641	7.091	1.00	40.00	7
	45	ATOM	38	CZ			692	-41.898	92.758	5.763	1.00	40.00	6
	73		39				692	-40.784	92.695	5.024		40.00	7
		ATOM			ARG			-43.034	92.940	5.080		40.00	7
		MOTA	40				692	-39.941	92.547	11.995		40.00	6
		ATOM	41	С					92.440	12.649		40.00	8
	50	ATOM	42	0			692	-41.001	92.440	11.816		40.00	7
	50	ATOM	43	N			693	-39.095				40.00	6
		ATOM	44	CA			693	-39.230	90.232	12.395			
		MOTA	45	CB			693	-38.362	89.337	11.615		40.00	6
		MOTA	46	CG			693	-38.737	89.375	10.132		40.00	6
		ATOM	47				693	-37.794	88.570	9.247		40.00	6
	55	ATOM	48	CD2	LEU	Y	693	-40.142	88.827	9.862		40.00	6
		MOTA	49	С	LEU	Y	693	-38.921	90.378	13.816		40.00	6
		ATOM	50	0	LEU	Y	693	-39.191	89.474	14.615		40.00	8
		ATOM	51	N	LEU	Y	694	-38.366	91.533	14.076	1.00	40.00	7

ATOM	52	CA	LEU '	Y	694	_	38.174	91.885	15.435	1.00	40.00	6
		СВ				_	37.181	93.002	15.561			6
	54	CG	LEU .	Y	694	-	35.799	92.377	15.869	1.00	40.00	6
	55	CD1	LEU '	Y	694	_	34.897	93.275	16.702	1.00	40.00	6
ATOM	56	CD2	LEU	Y	694	_	35.897	91.055	16.661	1.00		6
ATOM	57	С	LEU	Y	694	_	39.596	91.903				6
ATOM	58	0	LEU	Y	694	_	39.985	91.253				8
ATOM	59	N	GLN	Y	695	-	40.787	92.229				7
ATOM	60	CA	GLN	Y	695	_	42.034	91.457				6
ATOM	61	С	GLN	Y	695	_	43.054	90.901				6
ATOM	62	0	GLN	Y	695	_	43.102	91.557				8
MOTA	63	CB	GLN	Y	695	_	42.362					6
ATOM	64	CG	GLN	Y	695							6
MOTA	65	CD	GLN	Y	695							6
ATOM	66	OE1	GLN	Y	695	-	41.828					8
ATOM	67	NE2	GLN	Y	695							7
ATOM	68	N	ASP	Y	696							7
ATOM	69	CA	ASP	Y	696	_	44.784					6
ATOM	70	С	ASP	Y	696	-	46.034					6
ATOM	71	0	ASP	Y	696							8
ATOM	72	CB	ASP	Y	696							6
MOTA	73	CG	ASP	Y	696	-	44.021					6
MOTA	74	OD1	ASP	Y	696							8
MOTA	75	OD2	ASP	Y	696	-	-44.212	85.591	15.889	1.00	40.00	8
END												
	ATOM ATOM ATOM ATOM ATOM ATOM ATOM ATOM	ATOM 53 ATOM 54 ATOM 55 ATOM 56 ATOM 56 ATOM 57 ATOM 58 ATOM 60 ATOM 61 ATOM 62 ATOM 63 ATOM 64 ATOM 65 ATOM 66 ATOM 67 ATOM 68 ATOM 67 ATOM 68 ATOM 70 ATOM 70 ATOM 71 ATOM 72 ATOM 73 ATOM 74 ATOM 75	ATOM 53 CB ATOM 54 CG ATOM 55 CD1 ATOM 56 CD2 ATOM 57 C ATOM 58 O ATOM 59 N ATOM 60 CA ATOM 61 C ATOM 62 O ATOM 63 CB ATOM 65 CD ATOM 66 OE1 ATOM 66 OE1 ATOM 67 NE2 ATOM 68 N ATOM 69 CA ATOM 70 C ATOM 71 O ATOM 72 CB ATOM 73 CG ATOM 74 OD1 ATOM 75 OD2	ATOM 53 CB LEU ATOM 54 CG LEU ATOM 55 CD1 LEU ATOM 56 CD2 LEU ATOM 57 C LEU ATOM 58 O LEU ATOM 59 N GLN ATOM 60 CA GLN ATOM 61 C GLN ATOM 63 CB GLN ATOM 64 CG GLN ATOM 65 CD GLN ATOM 66 OE1 GLN ATOM 67 NE2 GLN ATOM 68 N ASP ATOM 70 C ASP ATOM 71 O ASP ATOM 72 CB ASP ATOM 73 CG ASP ATOM 74 OD1 ASP ATOM 74 OD1 ASP ATOM 75 OD2 ASP	ATOM 53 CB LEU Y ATOM 54 CG LEU Y ATOM 55 CD1 LEU Y ATOM 56 CD2 LEU Y ATOM 57 C LEU Y ATOM 58 O LEU Y ATOM 59 N GLN Y ATOM 60 CA GLN Y ATOM 61 C GLN Y ATOM 62 O GLN Y ATOM 63 CB GLN Y ATOM 64 CG GLN Y ATOM 65 CD GLN Y ATOM 66 OE1 GLN Y ATOM 66 OE1 GLN Y ATOM 67 NE2 GLN Y ATOM 68 N ASP Y ATOM 69 CA ASP Y ATOM 70 C ASP Y ATOM 71 O ASP Y ATOM 72 CB ASP Y ATOM 73 CG ASP Y ATOM 74 OD1 ASP Y ATOM 74 OD1 ASP Y	ATOM 53 CB LEU Y 694 ATOM 54 CG LEU Y 694 ATOM 55 CD1 LEU Y 694 ATOM 56 CD2 LEU Y 694 ATOM 57 C LEU Y 694 ATOM 58 O LEU Y 694 ATOM 59 N GLN Y 695 ATOM 60 CA GLN Y 695 ATOM 61 C GLN Y 695 ATOM 62 O GLN Y 695 ATOM 63 CB GLN Y 695 ATOM 64 CG GLN Y 695 ATOM 65 CD GLN Y 695 ATOM 66 OE1 GLN Y 695 ATOM 66 OE1 GLN Y 695 ATOM 67 NE2 GLN Y 695 ATOM 68 N ASP Y 696 ATOM 69 CA ASP Y 696 ATOM 70 C ASP Y 696 ATOM 71 O ASP Y 696 ATOM 72 CB ASP Y 696 ATOM 73 CG ASP Y 696 ATOM 74 OD1 ASP Y 696 ATOM 75 OD2 ASP Y 696	ATOM 53 CB LEU Y 694 ATOM 54 CG LEU Y 694 ATOM 55 CD1 LEU Y 694 ATOM 56 CD2 LEU Y 694 ATOM 57 C LEU Y 694 ATOM 58 O LEU Y 694 ATOM 59 N GLN Y 695 ATOM 60 CA GLN Y 695 ATOM 61 C GLN Y 695 ATOM 62 O GLN Y 695 ATOM 63 CB GLN Y 695 ATOM 64 CG GLN Y 695 ATOM 65 CD GLN Y 695 ATOM 66 OE1 GLN Y 695 ATOM 66 OE1 GLN Y 695 ATOM 67 NE2 GLN Y 695 ATOM 68 N ASP Y 696 ATOM 70 C ASP Y 696 ATOM 71 O ASP Y 696 ATOM 72 CB ASP Y 696 ATOM 73 CG ASP Y 696 ATOM 74 OD1 ASP Y 696	ATOM 53 CB LEU Y 694 -37.181 ATOM 54 CG LEU Y 694 -35.799 ATOM 55 CD1 LEU Y 694 -34.897 ATOM 56 CD2 LEU Y 694 -35.897 ATOM 57 C LEU Y 694 -39.596 ATOM 58 O LEU Y 694 -39.985 ATOM 59 N GLN Y 695 -40.787 ATOM 60 CA GLN Y 695 -42.034 ATOM 61 C GLN Y 695 -43.054 ATOM 62 O GLN Y 695 -43.102 ATOM 63 CB GLN Y 695 -42.362 ATOM 64 CG GLN Y 695 -41.013 ATOM 65 CD GLN Y 695 -41.013 ATOM 66 OE1 GLN Y 695 -40.943 ATOM 67 NE2 GLN Y 695 -39.938 ATOM 68 N ASP Y 696 -44.784 ATOM 69 CA ASP Y 696 -44.784 ATOM 70 C ASP Y 696 -46.034 ATOM 71 O ASP Y 696 -45.211 ATOM 73 CG ASP Y 696 -44.021 ATOM 74 OD1 ASP Y 696 -44.212	ATOM 53 CB LEU Y 694 -37.181 93.002 ATOM 54 CG LEU Y 694 -35.799 92.377 ATOM 55 CD1 LEU Y 694 -34.897 93.275 ATOM 56 CD2 LEU Y 694 -35.897 91.055 ATOM 57 C LEU Y 694 -39.596 91.903 ATOM 58 O LEU Y 694 -39.985 91.253 ATOM 59 N GLN Y 695 -40.787 92.229 ATOM 60 CA GLN Y 695 -42.034 91.457 ATOM 61 C GLN Y 695 -43.054 90.901 ATOM 62 O GLN Y 695 -43.102 91.557 ATOM 63 CB GLN Y 695 -42.362 92.025 ATOM 64 CG GLN Y 695 -41.013 92.101 ATOM 65 CD GLN Y 695 -40.943 91.235 ATOM 66 OE1 GLN Y 695 -41.828 90.426 ATOM 67 NE2 GLN Y 695 -39.938 91.399 ATOM 68 N ASP Y 696 -43.802 89.498 ATOM 69 CA ASP Y 696 -44.784 88.354 ATOM 70 C ASP Y 696 -46.034 88.934 ATOM 71 O ASP Y 696 -46.266 88.655 ATOM 72 CB ASP Y 696 -44.021 86.560 ATOM 74 OD1 ASP Y 696 -44.212 85.591	ATOM 53 CB LEU Y 694 -37.181 93.002 15.561 ATOM 54 CG LEU Y 694 -35.799 92.377 15.869 ATOM 55 CD1 LEU Y 694 -34.897 93.275 16.702 ATOM 56 CD2 LEU Y 694 -35.897 91.055 16.661 ATOM 57 C LEU Y 694 -39.596 91.903 15.915 ATOM 58 O LEU Y 694 -39.985 91.253 16.858 ATOM 59 N GLN Y 695 -40.787 92.229 15.048 ATOM 60 CA GLN Y 695 -42.034 91.457 15.543 ATOM 61 C GLN Y 695 -43.054 90.901 14.240 ATOM 62 O GLN Y 695 -43.102 91.557 13.189 ATOM 63 CB GLN Y 695 -42.362 92.025 16.923 ATOM 64 CG GLN Y 695 -41.013 92.101 17.768 ATOM 65 CD GLN Y 695 -40.943 91.235 19.059 ATOM 66 OE1 GLN Y 695 -41.828 90.426 19.318 ATOM 67 NE2 GLN Y 695 -39.938 91.399 19.916 ATOM 68 N ASP Y 696 -43.802 89.498 14.402 ATOM 69 CA ASP Y 696 -44.784 88.354 13.428 ATOM 70 C ASP Y 696 -46.034 88.934 12.759 ATOM 71 O ASP Y 696 -46.266 88.655 11.529 ATOM 72 CB ASP Y 696 -44.021 86.560 15.058 ATOM 74 OD1 ASP Y 696 -42.823 86.994 14.844 ATOM 75 OD2 ASP Y 696 -44.212 85.591 15.889	ATOM 53 CB LEU Y 694	ATOM 53 CB LEU Y 694 -37.181 93.002 15.561 1.00 40.00 ATOM 54 CG LEU Y 694 -35.799 92.377 15.869 1.00 40.00 ATOM 55 CD1 LEU Y 694 -34.897 93.275 16.702 1.00 40.00 ATOM 56 CD2 LEU Y 694 -35.897 91.055 16.661 1.00 40.00 ATOM 57 C LEU Y 694 -39.596 91.903 15.915 1.00 40.00 ATOM 58 O LEU Y 694 -39.985 91.253 16.858 1.00 40.00 ATOM 59 N GLN Y 695 -40.787 92.229 15.048 1.00 40.00 ATOM 60 CA GLN Y 695 -42.034 91.457 15.543 1.00 40.00 ATOM 61 C GLN Y 695 -43.054 90.901 14.240 1.00 40.00 ATOM 62 O GLN Y 695 -43.102 91.557 13.189 1.00 40.00 ATOM 63 CB GLN Y 695 -42.362 92.025 16.923 1.00 40.00 ATOM 65 CD GLN Y 695 -41.013 92.101 17.768 1.00 40.00 ATOM 66 OE1 GLN Y 695 -41.013 92.101 17.768 1.00 40.00 ATOM 66 OE1 GLN Y 695 -41.828 90.426 19.318 1.00 40.00 ATOM 67 NE2 GLN Y 695 -39.938 91.399 19.916 1.00 40.00 ATOM 68 N ASP Y 696 -43.802 89.498 14.402 1.00 40.00 ATOM 68 N ASP Y 696 -43.802 89.498 14.402 1.00 40.00 ATOM 60 CA ASP Y 696 -44.784 88.354 13.428 1.00 40.00 ATOM 67 NE2 GLN Y 695 -44.784 88.354 13.428 1.00 40.00 ATOM 67 NE2 GLN Y 695 -44.784 88.354 13.428 1.00 40.00 ATOM 67 C ASP Y 696 -46.034 88.934 12.759 1.00 40.00 ATOM 70 C ASP Y 696 -46.034 88.934 12.759 1.00 40.00 ATOM 71 O ASP Y 696 -46.266 88.655 11.529 1.00 40.00 ATOM 72 CB ASP Y 696 -46.266 88.655 11.529 1.00 40.00 ATOM 73 CG ASP Y 696 -46.266 88.655 11.529 1.00 40.00 ATOM 74 OD1 ASP Y 696 -44.021 86.560 15.058 1.00 40.00 ATOM 74 OD1 ASP Y 696 -44.2823 86.994 14.844 1.00 40.00 ATOM 74 OD1 ASP Y 696 -44.2823 86.994 14.844 1.00 40.00 ATOM 74 OD1 ASP Y 696 -42.823 86.994 14.844 1.00 40.00 ATOM 75 OD2 ASP Y 696 -44.2823 86.994 14.844 1.00 40.00 ATOM 75 OD2 ASP Y 696 -44.2823 86.994 14.844 1.00 40.00 ATOM 75 OD2 ASP Y 696 -44.2823 86.994 14.844 1.00 40.00 ATOM 75 OD2 ASP Y 696 -44.212 85.591 15.889 1.00 40.00

Atomic Coordinates for Human ERα Complexed with DES, and a GRIP1 NR-box 2 Peptide

Atomic Cooldinates for Human Erec Completed W122223													-	
	10	CRYST1	54.094	82	.217	58.	041	90.00	111	1.33 9	0.00	P	21	2
		ORIGX1	1.00	0000	0.000	000	0.0	00000	0.0	00000				
		ORIGX2	0.00		1.000		0.0	00000	0.0	00000				
		ORIGX3	0.00		0.000	000	1.0	00000	0.0	00000				
		SCALE1	0.01		0.000	000	0.0	07221	0.	00000				
	15	SCALE2	0.00		0.012		0.0	00000	0.	00000				
	10	SCALE3	0.00		0.000		0.0	18497	0.	00000				
		301												
		ATOM	1	CB	SER	A	305			-14.787			.00	73.26
		MOTA	2	C	SER	A	305			-14.303			.00	72.95
	20	MOTA	3	0	SER	Α	305			-13.984			.00	72.46
		MOTA	4	N	SER	A	305			-16.033			.00	74.06
		MOTA		CA	SER	A	305			-14.713			.00	73.59 72.21
		MOTA	6	N	LEU	A	306			-14.313			.00	72.21
.:025		MOTA	7	CA	LEU	A	306			-13.950			00	70.19
	25	MOTA	8	CB	LEU	A	306			-14.256			00	69.57
		MOTA	9	C	LEU	A	306			-12.478			00	69.96
14 ***		MOTA	10	0	LEU	A	306			-11.638			.00	68.06
IJ.		MOTA		N	ALA	A	307			-12.176			00	
		MOTA	12	CA	ALA	A.	307			-10.810			00	
	30	MOTA	13	CB	ALA	A	307			-10.795 -10.204			00	
Branchine No. 5		ATOM	14	C	ALA	A	307		. 192	-8.984			.00	
		MOTA	15	0	ALA	A	307			-11.06			1.00	
		MOTA	16	N	LEU	A	308 308		.487				.00	
1.1	٥.	MOTA	17	CA	LEU	A	308		.423	-11.74			.00	
	35	ATOM	18	CB	LEU LEU	A A	308			-12.68			1.00	
		ATOM	19	CG CD1	LEU	A	308		.188		3 10.40		L.00	
		MOTA	20 21	CD1	LEU	A	308		.919				L.00	
:#		ATOM ATOM	22	CDZ	LEU	A	308		.903	-10.03			L.00	61.61
1 4	40	ATOM	23	0	LEU	A	308		.385	-9.44		6 :	L.00	62.92
	40	MOTA	24	N	SER	A	309		.561	-10.21	9 6.95	9 1	1.00	60.50
		ATOM	25	CA	SER	A	309	37	.928	-9.74	3 6.77	1 :	1.00	
		ATOM	26	CB	SER	A	309	38	.720	-10.75			1.00	
		ATOM	27	OG	SER	A	309	38	.889				1.00	
	45	ATOM	28	С	SER	Α	309		.986	-8.37			1.00	
		ATOM	29	0	SER	A	309		.965	-7.63			1.00	
		MOTA	30	N	LEU	Α	310		.940	-8.03			1.00	
		ATOM	31	CA	LEU	Α	310		.877	-6.75			1.00	
		MOTA	32	CB	LEU	Α	310		5.516	-6.59			1.00	
	50	MOTA	33	CG	LEU	A	310		.301				1.00	
		ATOM	34	CD1	LEU	A	310		.951				1.00	
		ATOM	35	CD2	LEU	A	310		5.417				1.00 1.00	
		MOTA	36	C	LEU	A	310		7.086					
		MOTA	37	0	LEU	A	310		5.605				1.00 1.00	
	55	MOTA	38	N	THR	A	311		7.812				1.00	
		MOTA	39	CA	THR	A	311		3.034				1.00	
		ATOM	40	CB	THR	A	311		9.313				1.00	
		MOTA	41	OG1	THR	A	311		9.079				1.00	
		MOTA	42	CG2	THR	A	311		0.464 5.834				1.00	
	60	MOTA	43	C	THR	A n	311 311		5.034 5.021				1.00	
		ATOM	44	0	THR	A	211	31	J . UZII			-		

	5	ATOM	103	CB	LEU	A	320	30.052	1.839 -4.155	1.00	33.52
		ATOM	104	CG	LEU	A	320	29.974	2.899 -3.054	1.00	34.60
		MOTA	105	CD1	LEU	A	320	31.060	3.940 -3.292	1.00	33.69
		ATOM	106	CD2	LEU	A	320	28.611	3.562 -3.044	1.00	31.05
		MOTA	107	С	LEU	A	320	29.052	0.040 -5.561	1.00	35.41
	10	ATOM	108	0	LEU	A	320	28.230	0.271 -6.446	1.00	39.16
		ATOM	109	N	AASP	Α	321	30.042	-0.833 -5.720	0.50	36.33
		MOTA	110	N	BASP	A	321	30.041	-0.839 -5.695	0.50	35.76
		ATOM	111	CA	AASP	A	321	30.214	-1.559 -6.977	0.50	37.71
		ATOM	112	CA	BASP	A	321	30.258	-1.595 -6.925	0.50	37.11
	15	ATOM	113	CB	AASP	A	321	31.537	-2.334 -6.973	0.50	40.01
		MOTA	114	CB	BASP	A	321	31.573	-2.374 -6.826	0.50	39.41
		ATOM	115	CG	AASP	A	321	31.694	-3.230 -8.195	0.50	41.93
		ATOM	116	CG	BASP	A	321	32.770	-1.562 -7.284	0.50	39.96
		ATOM	117	OD1	AASP	A	321	31.523	-2.733 -9.329	0.50	42.11
	20	ATOM	118	OD1	BASP	A	321	33.312	-1.868 -8.366	0.50	43.41
		ATOM	119	OD2	AASP	A	321	31.988	-4.432 -8.022	0.50	42.69
		ATOM	120	OD2	BASP	A	321	33.170	-0.622 -6.564	0.50	41.33
		ATOM	121	C	AASP	A	321	29.069	-2.524 -7.275	0.50	37.19
		ATOM	122	C	BASP	A	321	29.123	-2.565 -7.253	0.50	36.68
	25	ATOM	123	0	AASP	A	321	28.820	-2.861 -8.434	0.50	36.87
		ATOM	124	0	BASP	A	321	28.934	-2.942 -8.411	0.50	36.08
IU		ATOM	125	N	ALA	A	322	28.374	-2.968 -6.235	1.00	35.35
		ATOM	126	CA	ALA	A	322	27.268	-3.902 -6.417	1.00	31.59
		ATOM	127	CB	ALA	A	322	27.124	-4.781 -5.175	1.00	30.73
-	30	ATOM	128	C	ALA	A	322	25.946	-3.204 -6.709	1.00	30.07
		ATOM	129	0	ALA	A	322	24.955	-3.857 -7.036	1.00	26.53
1		ATOM	130	N	GLU	A	323	25.932	-1.880 -6.596	1.00	27.98
		ATOM	131	CA	GLU	A	323	24.713	-1.117 -6.827	1.00	29.88
		ATOM	132	CB	GLU	A	323	25.027	0.380 -6.855	1.00	30.98
	35	ATOM	133	CG	GLU	A	323	24.870	1.068 -5.509	1.00	31.62
4.5-22		ATOM	134	CD	GLU	A	323	23.463	0.940 -4.960	1.00	31.98
		ATOM	135	OE1	GLU	A	323	23.183	-0.056 -4.257	1.00	33.10
ind . FR		ATOM	136	OE2	GLU	A	323	22.640	1.836 -5.233	1.00	30.01
		ATOM	137	C	GLU	A	323	24.010	-1.515 -8.123	1.00	30.86
	40	ATOM	138	Ō	GLU	A	323	24.655	-1.705 -9.151	1.00	28.86
		ATOM	139	N	PRO	A	324	22.674	-1.659 -8.083	1.00	30.66
		ATOM	140	CD	PRO	Α	324	21.774	-1.466 -6.935	1.00	31.01
		ATOM	141	CA	PRO	A	324	21.935	-2.032 -9.290	1.00	30.29
		ATOM	142	CB	PRO	A	324	20.613	-2.598 -8.760	1.00	31.42
	45	ATOM	143	CG	PRO	A	324	20.626	-2.363 -7.258	1.00	33.66
		ATOM	144	C	PRO	A	324	21.717	-0.785-10.138	1.00	27.46
		ATOM	145	0	PRO	Α	324	21.893	0.332 -9.668	1.00	26.19
		ATOM	146	N	PRO	A	325	21.335	-0.959-11.403	1.00	27.80
		ATOM	147	CD	PRO	A	325	21.082	-2.198-12.161	1.00	27.35
	50	ATOM	148	CA	PRO	A	325	21.125	0.242-12.211	1.00	25.59
	-	ATOM	149	CB	PRO	A	325	21.258	-0.266-13.637	1.00	24.02
		ATOM	150	CG	PRO	A	325	20.773	-1.695-13.559	1.00	26.00
		ATOM	151	C	PRO	A	325	19.749	0.830-11.954	1.00	23.73
		ATOM	152	0	PRO	A	325	18.873	0.165-11.402	1.00	24.83
	55	ATOM	153	N	ILE	A	326	19.571	2.081-12.352	1.00	22.11
		ATOM	154	CA	ILE	A	326	18.296	2.762-12.212	1.00	24.01
		ATOM	155	CB	ILE	A	326	18.502	4.282-12.133	1.00	25.97
		ATOM	156	CG2	ILE	A	326	17.168	4.992-12.286	1.00	20.75
		ATOM	157	CG1	ILE	A	326	19.189	4.632-10.805	1.00	29.31
	60	ATOM	158	CD1	ILE	A	326	19.301	6.120-10.525	1.00	32.91
	50	ATOM	159	C	ILE	A	326	17.506	2.408-13.471	1.00	25.72
		ATOM	160	0	ILE	A	326	17.906	2.758-14.581	1.00	25.55
		-11 01.1	_ 0	_	انبه سد عد		220	-/.500	2.700 14.001		

	5	ATOM	161	N	LEU	A	327	16.392	1.703-13.301	1.00	25.57
	5	ATOM	162	CA	LEU	A	327	15.595	1.279-14.439	1.00	23.80
			163	CB	LEU	A	327	14.872		1.00	23.96
		ATOM		CG	LEU	A	327	15.778		1.00	19.89
		ATOM	164		LEU	A	327	14.944		1.00	21.19
	10	ATOM	165	CD1		A	327	16.850	-1.415-14.805	1.00	17.53
	10	MOTA	166	CD2	LEU		327	14.598		1.00	27.16
		ATOM	167	C	LEU	A		14.161	3.202-14.194	1.00	25.98
		ATOM	168	0	LEU	A	327		2.207-16.210	1.00	26.56
		ATOM	169	N	TYR	A	328	14.251	3.123-16.814	1.00	24.45
		ATOM	170	CA	TYR	A	328	13.303	3.465-18.245	1.00	26.72
	15	ATOM	171	CB	TYR	A	328	13.724	4.693-18.314	1.00	27.73
		ATOM	172	CG	TYR	A	328	14.587		1.00	28.56
		ATOM	173	CD1	TYR	A	328	14.021	5.949-18.518		29.10
		MOTA	174	CE1	TYR	A	328	14.798	7.092-18.509	1.00	26.01
		MOTA	175	CD2	TYR	A	328	15.962	4.612-18.110	1.00	30.63
	20	MOTA	176	CE2	TYR	A	328	16.750	5.753-18.098	1.00	
		ATOM	177	CZ	TYR	A	328	16.157	6.988-18.297	1.00	30.07
		ATOM	178	OH	TYR	Α	328	16.917	8.130-18.265	1.00	37.94
		MOTA	179	С	TYR	A	328	11.923	2.501-16.827	1.00	24.95
		MOTA	180	0	TYR	A	328	11.774	1.274-16.846	1.00	27.02
	25	MOTA	181	N	SER	A	329	10.912	3.358-16.800	1.00	25.60
.31		MOTA	182	CA	SER	A	329	9.533	2.908-16.837	1.00	29.45
IJ		ATOM	183	CB	SER	Α	329	8.661	3.858-16.020	1.00	30.80
ij		MOTA	184	OG	SER	A	329	7.297	3.721-16.364	1.00	33.74
[-£		ATOM	185	C	SER	A	329	9.129	2.947-18.313	1.00	31.30
	30	ATOM	186	0	SER	A	329	9.908	3.397-19.154	1.00	27.35
		MOTA	187	N	GLU	A	330	7.930	2.469-18.629	1.00	32.98 35.10
إيد		MOTA	188	CA	GLU	A	330	7.459	2.482-20.007	1.00	34.67
#		MOTA	189	CB	GLU	A	330	6.031	1.968-20.074	1.00	40.06
		MOTA	190	С	GLU	A	330	7.532	3.924-20.505	1.00	40.00
	35	MOTA	191	0	GLU	A	330	7.068	4.841-19.826	1.00	41.16
		MOTA	192	N	TYR	A	331	8.124	4.126-21.681	1.00	42.66
		MOTA	193	CA	TYR	A	331	8.263	5.470-22.234 5.482-23.350	1.00	42.54
ıİ		MOTA	194	CB	TYR	A	331	9.323		1.00	38.67
: []		MOTA	195	CG	TYR	A	331	9.202	4.347-24.345 3.284-24.334	1.00	34.66
-20	40	ATOM	196	CD1	TYR	A	331	10.105	2.228-25.233	1.00	34.89
		MOTA	197	CE1	TYR	A	331	9.985	4.327-25.287	1.00	37.88
		MOTA	198	CD2	TYR	A	331	8.174	3.276-26.193	1.00	34.65
		ATOM	199	CE2	TYR	A	331	8.045 8.950	2.232-26.159	1.00	30.73
		ATOM	200	CZ	TYR	A	331	8.814	1.191-27.042	1.00	30.97
	45	MOTA	201	OH	TYR	A.	331	6.943	6.043-22.754	1.00	46.24
		ATOM	202	С	TYR	A	331	6.018	5.301-23.096	1.00	45.38
		MOTA	203	0	TYR	A	331	6.868	7.372-22.792	1.00	49.11
		ATOM	204	N	ASP	A	332	5.684	8.092-23.262	1.00	52.40
	~ o	ATOM	205	CA	ASP	A	332	5.781	8.321-24.772	1.00	52.86
	50	ATOM	206	CB	ASP	A	332	4.356	7.410-22.926	1.00	52.90
		MOTA	207	C	ASP	A	332	3.561	7.116-23.818	1.00	53.94
		MOTA	208	0	ASP	A	332	4.103	7.144-21.632	1.00	53.63
		MOTA	209	N	PRO	A	333	4.962	7.418-20.465	1.00	53.63
	~ ~	MOTA	210	CD	PRO	A	333 333	2.840	6.497-21.253	1.00	53.55
	55	MOTA	211	CA	PRO	A		3.070	6.076-19.802	1.00	53.78
		MOTA	212	CB	PRO	A	333 333	4.101	7.028-19.290	1.00	53.42
		MOTA	213	CG	PRO	A.	333	1.673	7.478-21.398	1.00	52.17
		MOTA	214	C	PRO	A A	333	1.879	8.690-21.395	1.00	51.19
	<i>(</i> 0	MOTA	215	O NT	PRO TUD	A	334	0.457	6.956-21.532	1.00	52.26
	60	ATOM	216	N	${ m THR}$	A	334	-0.724	7.802-21.687	1.00	54.21
		ATOM	217 218	CA CB	THR	A	334	-1.997	6.949-21.813	1.00	53.90
		ATOM	210	CD	1111	23	231	153			

					_		4 054	6.256-23.065	1.00	53.92
5	MOTA	219	OG1	THR	A	334	-1.971		1.00	54.15
	MOTA	220	CG2	THR	A	334	-3.237	7.821-21.761		
	MOTA	221	С	THR	A	334	-0.864	8.782-20.525	1.00	56.34
	ATOM	222	0	THR	A	334	-1.389	8.443-19.461	1.00	56.44
	ATOM	223	N	ARG	Α	335	-0.386	10.002-20.766	1.00	58.24
10	ATOM	224	CA	ARG	A	335	-0.377	11.099-19.801	1.00	57.96
	MOTA	225	CB	ARG	Α	335	-0.569	12.427-20.531	1.00	60.22
	ATOM	226	C	ARG	A	335	-1.349	10.996-18.627	1.00	56.61
	ATOM	227	0	ARG	A	335	-0.919	10.908-17.475	1.00	60.70
	ATOM	228	N	PRO	A	336	-2.667	11.015-18.889	1.00	52.43
15	ATOM	229	CD	PRO	A	336	-3.389	11.117-20.165	1.00	49.06
13	ATOM	230	CA	PRO	A	336	-3.587	10.915-17.752	1.00	49.58
			CB	PRO	A	336	-4.911	11.456-18.302	1.00	48.66
	ATOM	231		PRO	A	336	-4.645	11.809-19.760	1.00	51.33
	ATOM	232	CG			336	-3.698	9.468-17.279	1.00	49.25
	MOTA	233	C	PRO	A		-4.340	8.644-17.929	1.00	48.06
20	MOTA	234	0	PRO	A -	336		9.170-16.147	1.00	47.90
	MOTA	235	N	PHE	A	337	-3.063		1.00	46.61
	MOTA	236	CA	PHE	A	337	-3.055	7.821-15.582	1.00	47.73
	ATOM	237	CB	PHE	A	337	-2.063	7.732-14.421		46.27
	MOTA	238	CG	PHE	A	337	-0.649	8.011-14.805	1.00	
5 25	MOTA	239	CD1	PHE	A	337	-0.017	9.168-14.368	1.00	46.55
Character Character Transport To the Control of the	MOTA	240	CD2	PHE	Α	337	0.061	7.113-15.591	1.00	48.12
	MOTA	241	CE1	PHE	A	337	1.305	9.429-14.707	1.00	48.09
in fager	ATOM	242	CE2	PHE	Α	337	1.386	7.364-15.938	1.00	47.57
	MOTA	243	CZ	PHE	Α	337	2.009	8.525-15.495	1.00	48.40
30	ATOM	244	С	PHE	Α	337	-4.401	7.338-15.071	1.00	46.15
	MOTA	245	0	PHE	A	337	-5.250	8.127-14.671	1.00	48.34
	ATOM	246	N	SER	A	338	-4.573	6.022-15.080	1.00	45.06
	ATOM	247	CA	SER	A	338	-5.781	5.385-14.578	1.00	45.12
##	ATOM	248	CB	SER	A	338	-6.477	4.594-15.684	1.00	44.49
3 5	ATOM	249	OG	SER	A	338	-6.227	3.206-15.554	1.00	45.78
11 33		2 4 9 250	C	SER	A	338	-5.292	4.439-13.488	1.00	47.04
ii ii ii ii ii ii ii ii ii ii ii ii ii i	MOTA		0	SER	A	338	-4.090	4.186-13.387	1.00	44.08
	ATOM	251	N	GLU	A	339	-6.206	3.916-12.676	1.00	45.63
	ATOM	252		GLU	A	339	-5.802	3.012-11.608	1.00	45.40
1	MOTA	253	CA			339	-7.015	2.521-10.814	1.00	45.66
40	MOTA	254	CB	GLU	A		-6.637	1.680 -9.600	1.00	46.81
	MOTA	255	CG	GLU	A	339	-7.717	1.652 -8.535	1.00	47.56
	ATOM	256	CD	GLU	A	339		0.656 -8.477	1.00	47.37
	MOTA	257	OE1	GLU	A	339	-8.471		1.00	49.29
	MOTA	258	OE2	GLU	A	339	-7.810	2.625 -7.754	1.00	45.23
45	MOTA	259	С	GLU	A	339	-5.040	1.821-12.170		
	MOTA	260	0	GLU	A	339	-3.862	1.641-11.872	1.00	46.51
	ATOM	261	N	ALA	Α	340	-5.712	1.010-12.982	1.00	42.87
	ATOM	262	CA	ALA	A	340	-5.078	-0.158-13.574	1.00	40.24
	ATOM	263	CB	ALA	Α	340	-6.055	-0.871-14.496	1.00	41.40
50	MOTA	264	C	ALA	Α	340	-3.837	0.273-14.350	1.00	38.83
	ATOM	265	0	ALA	Α	340	-2.909	-0.515-14.543	1.00	35.58
	ATOM	266	N	SER	Α	341	-3.836	1.535-14.773	1.00	35.79
	ATOM	267	CA	SER	А	341	-2.742	2.133-15.537	1.00	36.58
	MOTA	268	CB	SER	A	341	-3.231	3.454-16.154	1.00	39.01
55		269	OG	SER	A	341	-2.211	4.130-16.864	1.00	36.09
33	ATOM				A	341	-1.480	2.376-14.691	1.00	35.63
	ATOM	270	C	SER		341	-0.389	1.913-15.038		33.20
	MOTA	271	0	SER	A n		-1.626	3.115-13.595		35.92
	MOTA	272	N	MET	A	342	-0.498	3.396-12.708		35.88
	MOTA	273	CA	MET	A	342	-0.498	4.396-11.623		
60	MOTA	274	CB	MET	A	342		5.218-11.059		
	MOTA	275	CG	MET	A	342	0.241	6.374 -9.780	1.00	44.73
	MOTA	276	SD	MET	A	342	-0.308	0.3/4 -7./60	1.00	·/J
							15/			

_					_	2.40	0 606	7.815-10.205	1.00	42.49
5	MOTA	277	CE	MET	A	342	0.626		1.00	34.17
	MOTA	278	C	\mathtt{MET}	Α	342	-0.011	2.100-12.059		
	MOTA	279	0	MET	A	342	1.195	1.880-11.909	1.00	33.40
	MOTA	280	N	MET	Α	343	-0.957	1.243-11.687	1.00	29.95
	MOTA	281	CA	MET	Α	343	-0.640	-0.034-11.062	1.00	31.96
10	MOTA	282	CB	MET	A	343	-1.921	-0.810-10.751	1.00	31.70
	MOTA	283	CG	MET	Α	343	-2.667	-0.337 -9.502	1.00	37.13
	ATOM	284	SD	MET	A	343	-1.749	-0.507 -7.940	1.00	36.00
	MOTA	285	CE	MET	A	343	-1.468	-2.299 -7.886	1.00	32.14
		286	C	MET	A	343	0.234	-0.875-11.979	1.00	31.72
1.5	MOTA		0	MET	A	343	1.159	-1.558-11.527	1.00	30.26
15	MOTA	287			A	344	-0.069	-0.823-13.272	1.00	29.04
	MOTA	288	N	GLY		344	0.688	-1.591-14.242	1.00	24.94
	MOTA	289	CA	GLY	A			-1.085-14.396	1.00	26.01
	MOTA	290	C	GLY	A	344	2.104	-1.873-14.463	1.00	28.72
	MOTA	291	0	GLY	A	344	3.046		1.00	26.97
20	MOTA	292	N	LEU	A	345	2.257	0.232-14.471		31.15
	MOTA	293	CA	LEU	А	345	3.576	0.839-14.608	1.00	
	MOTA	294	CB	LEU	Α	345	3.459	2.361-14.753	1.00	30.06
	MOTA	295	CG	LEU	Α	345	2.765	2.924-15.995	1.00	33.50
	ATOM	296	CD1	LEU	A	345	2.901	4.439-15.999	1.00	33.52
25	MOTA	297	CD2	LEU	A	345	3.379	2.324-17.257	1.00	33.22
	ATOM	298	С	LEU	A	345	4.433	0.534-13.383	1.00	30.31
	ATOM	299	0	LEU	A	345	5.564	0.061-13.505	1.00	32.80
14	ATOM	300	N	LEU	A	346	3.884	0.813-12.205	1.00	27.83
1 .	ATOM	301	CA	LEU	A	346	4.595	0.596-10.947	1.00	26.19
30	MOTA	302	CB	LEU	A	346	3.729	1.063 -9.783	1.00	24.51
· j 30		302	CG	LEU	A	346	3.483	2.569 -9.682	1.00	26.33
	ATOM	303	CD1	LEU	A	346	2.623	2.844 -8.463	1.00	27.33
To the state of th	MOTA		CD1	LEU	A	346	4.809	3.317 -9.587	1.00	24.89
¥!	ATOM	305				346	5.032	-0.848-10.707	1.00	25.72
5 35	MOTA	306	C	LEU	A.		6.181	-1.102-10.345	1.00	29.86
<u>⊯</u> 35	ATOM	307	0	LEU	A	346	4.117	-1.793-10.891	1.00	23.80
	MOTA	308	N	THR	A	347		-3.196-10.674	1.00	23.91
-	MOTA	309	CA	THR	A	347	4.436	-4.058-10.641	1.00	26.39
1000	MOTA	310	CB	THR	A	347	3.164		1.00	24.57
.5	MOTA	311	OG1	THR	A	347	2.421	-3.860-11.849	1.00	23.98
40	MOTA	312	CG2	THR	A	347	2.301	-3.682 -9.444		
	MOTA	313	С	THR	Α	347	5.366	-3.734-11.756	1.00	26.17
	MOTA	314	0	THR	A	347	6.176	-4.622-11.496	1.00	27.44
	MOTA	315	N	ASN	Α	348	5.242	-3.197-12.970	1.00	25.48
	MOTA	316	CA	ASN	Α	348	6.092	-3.617-14.082	1.00	23.77
45	MOTA	317	CB	ASN	Α	348	5.657	-2.926-15.385	1.00	24.59
	MOTA	318	CG	ASN	Α	348	6.522	-3.302-16.571	1.00	29.93
	MOTA	319	OD1	ASN	Α	348	7.616	-2.799-16.771	1.00	24.81
	MOTA	320	ND2	ASN	Α	348	6.010	-4.236-17.391	1.00	32.61
	ATOM	321	С	ASN	A	348	7.532	-3.229-13.741	1.00	22.82
50	ATOM	322	Ö	ASN	A	348	8.453	-4.027-13.870	1.00	18.83
50		323	N	LEU	A	349	7.711	-1.993-13.288	1.00	22.58
	MOTA		CA	LEU	A	349	9.030	-1.507-12.914	1.00	21.85
	ATOM	324				349	8.929	-0.028-12.536	1.00	22.00
	MOTA	325	CB	LEU	A		10.155	0.673-11.953	1.00	23.64
	MOTA	326	CG	LEU	A	349	11.224	0.826-13.017	1.00	19.35
55	MOTA	327	CD1	LEU	A	349		2.040-11.415	1.00	21.97
	MOTA	328	CD2	LEU	A	349	9.726	-2.335-11.734		22.94
	MOTA	329	С	LEU	A	349	9.564			23.97
	ATOM	330	0	LEU	A	349	10.724	-2.749-11.717		
	MOTA	331	N	ALA	A	350	8.705	-2.591-10.756	1.00	21.67
60	MOTA	332	CA	ALA	Α	350	9.113	-3.356 -9.586	1.00	21.83
	MOTA	333	CB	ALA	A	350	7.963	-3.441 -8.593	1.00	18.95
	ATOM	334	C	ALA	A	350	9.568	-4.757 -9.985	1.00	21.90
							155			

MOTA

					_		22.980 -17.173 4.110 1.00 41.42
5	ATOM	509	CB	LEU	A	370	
	ATOM	510	CG	LEU	A	370	
	MOTA	511	CD1	LEU	Α	370	22.219 -15.095 5.245 1.00 42.25
	ATOM	512	CD2	LEU	Α	370	23.931 -16.552 6.332 1.00 38.35
	MOTA	513	С	LEU	Α	370	23.449 -19.360 3.013 1.00 44.03
10	MOTA	514	0	LEU	Α	370	23.773 -19.423 1.829 1.00 43.63
10	ATOM	515	N	THR	Α	371	22.593 -20.206 3.575 1.00 44.29
	ATOM	516	CA	THR	A	371	21.968 -21.272 2.806 1.00 44.84
	ATOM	517	CB	THR	A	371	21.293 -22.302 3.730 1.00 45.65
		517	OG1	THR	A	371	20.262 -21.663 4.495 1.00 46.43
1.5	ATOM			THR	A	371	22.314 -22.903 4.677 1.00 46.48
15	MOTA	519	CG2			371	20.923 -20.684 1.864 1.00 44.93
	MOTA	520	C	THR	A		20.418 -19.585 2.092 1.00 44.36
	ATOM	521	0	THR	A	371	20.110
	MOTA	522	N	LEU	A	372	20.00,
	MOTA	523	CA	LEU	A	372	19.001
20	MOTA	524	CB	LEU	Α	372	13.107
	ATOM	525	CG	LEU	Α	372	10.512 21.030 1.01
	ATOM	526	CD1	LEU	Α	372	19.005 -20.417 -3.098 1.00 48.73
	MOTA	527	CD2	LEU	Α	372	18.521 -22.844 -3.420 1.00 51.12
	MOTA	528	С	LEU	A	372	18.307 -20.644 0.512 1.00 44.84
25	ATOM	529	0	LEU	Α	372	17.705 -19.602 0.261 1.00 43.25
	ATOM	530	N	HIS	A	373	17.849 -21.558 1.382 1.00 43.14
122	ATOM	531	CA	HIS	A	373	16.599 -21.353 2.100 1.00 42.23
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		532	CB	HIS	A	373	16.318 -22.525 3.062 1.00 45.38
5.4.3 5 .	ATOM	533	CG	HIS	A	373	15.114 -22.315 3.934 1.00 51.43
	MOTA			HIS	A	373	13.808 -22.621 3.743 1.00 54.99
30	MOTA	534	CD2			373	15.187 -21.716 5.174 1.00 54.26
<u> </u>	MOTA	535	ND1	HIS	A		13.979 -21.663 5.709 1.00 53.77
	MOTA	536	CE1	HIS	A	373	13.124 -22.206 4.861 1.00 55.27
#:	MOTA	537	NE2	HIS	A	373	
	ATOM	538	С	HIS	A	373	
35	MOTA	539	0	HIS	A	373	20.01
1.1	MOTA	540	N	ASP	A	374	27.002
<u> </u>	MOTA	541	CA	ASP	Α	374	10.010
	MOTA	542	CB	ASP	Α	374	19.287 -18.620 5.073 1.00 38.17
	MOTA	543	CG	ASP	Α	374	19.064 -19.425 6.344 1.00 41.47
40	ATOM	544	OD1	ASP	Α	374	17.896 -19.543 6.772 1.00 37.09
	ATOM	545	OD2	ASP	Α	374	20.052 -19.940 6.912 1.00 44.40
	MOTA	546	С	ASP	A	374	18.083 -17.277 3.326 1.00 37.19
	MOTA	547	0	ASP	Α	374	17.598 -16.208 3.696 1.00 38.13
	ATOM	548	N	GLN	Α	375	18.688 -17.431 2.152 1.00 33.13
45	ATOM	549	CA	GLN	А	375	18.788 -16.339 1.198 1.00 31.94
43	MOTA	550	CB	GLN	A	375	19.634 -16.756 -0.001 1.00 28.81
		551	CG	GLN	A	375	21.125 -16.570 0.189 1.00 31.71
	MOTA	552	CD	GLN	A	375	21.920 -17.222 -0.922 1.00 34.49
	ATOM			GLN	A	375	21.478 -17.267 -2.067 1.00 36.09
~ 0	MOTA	553	OE1			375	23.097 -17.736 -0.588 1.00 40.32
50	MOTA	554	NE2	GLN	A		17.379 -16.009 0.730 1.00 31.50
	MOTA	555	C	GLN	A	375	16.990 -14.840 0.653 1.00 27.42
	MOTA	556	0	GLN	A	375	20.330
	MOTA	557	N	VAL	A	376	10.01, 1.000
	MOTA	558	CA	VAL	Α	376	##
55	MOTA	559	CB	VAL	A	376	11.300
	MOTA	560	CG1	VAL	A	376	13.093 -18.122 -0.516 1.00 33.14
	MOTA	561	CG2	VAL	Α	376	15.232 -18.952 -1.485 1.00 30.79
	ATOM	562	С	VAL	A	376	14.393 -16.159 1.002 1.00 33.80
	MOTA	563	0	VAL	A	376	13.653 -15.237 0.661 1.00 34.89
60	MOTA	564	N	HIS	А	377	14.500 -16.568 2.261 1.00 33.35
00	ATOM	565	CA	HIS	A	377	13.730 -15.941 3.329 1.00 32.81
	MOTA	566	CB	HIS	А	377	13.966 -16.694 4.644 1.00 35.24
	VI OLI	500					150

	5	MOTA	567	CG	HIS	A	377	13.429	-15.989	5.851	1.00	40.15
		ATOM	568	CD2	HIS	A	377	14.054	-15.495	6.946	1.00	40.86
		MOTA	569	ND1	HIS	A	377		-15.703	6.012	1.00	43.08
		MOTA	570	CEl	HIS	A	377	11.913		7.154	1.00	42.44
		MOTA	571	NE2	HIS	A	377	13.089	-14.922	7.740	1.00	44.85
	10	ATOM	572	C	HIS	A	377	14.058	-14.454	3.507	1.00	28.63
		MOTA	573	0	HIS	Α	377	13.158	-13.619	3.613	1.00	29.20
		MOTA	574	N	LEU	A	378	15.343		3.544	1.00	24.41
		ATOM	575	CA	LEU	A	378	15.759	-12.738	3.721	1.00	23.21
	1.0	MOTA	576	CB	LEU	A	378	17.289		3.743	1.00	20.98
	15	MOTA	577	CG	LEU	A	378	17.960	-13.190	5.016	1.00	24.22
		MOTA	578	CD1	LEU	A	378	19.471	-13.041	4.924	1.00	21.07
		ATOM	579	CD2	LEU	A	378	17.431	-12.446	6.221	1.00	20.24
		MOTA	580	С	LEU	A	378	15.190	-11.827	2.630	1.00	24.78
		ATOM	581	0	LEU	Α	378	14.638	-10.766	2.922	1.00	22.09
	20	ATOM	582	N	LEU	A	379		-12.242	1.374	1.00	24.13
	20								-11.447	0.262	1.00	25.02
		ATOM	583	CA	LEU	A	379					
		ATOM	584	CB	LEU	A	379		-12.025		1.00	27.12
		MOTA	585	CG	LEU	Α	379		-11.600		1.00	24.39
		MOTA	586	CD1	$_{ m LEU}$	A	379	17.299	-12.557	-2.470	1.00	27.58
1200	25	MOTA	587	CD2	LEU	A	379	16.679	-10.178	-1.983	1.00	29.05
ij		ATOM	588	С	LEU	A	379	13.287	-11.355	0.246	1.00	27.61
		ATOM	589	0	LEU	A	379	12.726			1.00	26.16
II.		ATOM	590	N	GLU	A	380	12.616		0.576	1.00	25.65
i-s												
1	20	MOTA	591	CA	GLU	A	380	11.154		0.592	1.00	26.85
	30	ATOM	592	CB	GLU	A	380	10.640		0.871	1.00	29.38
i-i		MOTA	593	CG	GLU	A	380	10.718	-14.796	-0.331	1.00	35.58
i.		MOTA	594	CD	GLU	Α	380	10.228	-16.194	-0.025	1.00	39.31
E :		MOTA	595	OE1	GLU	A	380	10.142	-17.008	-0.967	1.00	42.89
		ATOM	596	OE2	GLU	A	380	9.927	-16.478	1.153	1.00	39.45
	35	ATOM	597	C	GLU	Α	380	10.604	-11.526	1.649	1.00	25.43
		MOTA	598	0	GLU	A	380	9.551	-10.925	1.469	1.00	27.75
i sing		ATOM	599	N	CYS	A	381	11.324		2.753	1.00	25.57
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1												
155		ATOM	600	CA	CYS	A -	381	10.907		3.843	1.00	26.46
	4.0	MOTA	601	CB	CYS	A	381	11.570	-11.000	5.149	1.00	31.46
	40	MOTA	602	SG	CYS	A	381	11.305	-9.946	6.623	1.00	45.32
		MOTA	603	C	CYS	A	381	11.262	-9.059	3.589	1.00	24.77
		ATOM	604	0	CYS	Α	381	10.516	-8.166	3.975	1.00	25.01
		MOTA	605	N	ALA	A	382	12.377	-8.815	2.903	1.00	22.23
		MOTA	606	CA	ALA	Α	382	12.855	-7.449	2.681	1.00	21.83
	45	ATOM	607	CB	ALA	A	382	14.319	-7.383	3.095	1.00	21.56
		ATOM	608	C	ALA	A	382	12.705	-6.778		1.00	19.78
										1.182		17.01
		MOTA	609	0	ALA	A	382	12.996	-5.587		1.00	
		ATOM	610	N	TRP	Α	383	12.261	-7.507		1.00	17.61
		MOTA	611	CA	TRP	A	383	12.164	-6.915		1.00	18.06
	50	MOTA	612	CB	TRP	A	383	11.580	-7.928	-2.035	1.00	20.28
		MOTA	613	CG	TRP	Α	383	10.105	-8.201	-1.919	1.00	20.50
		MOTA	614	CD2	TRP	Α	383	9.049	-7.509	-2.599	1.00	22.48
		ATOM	615	CE2	TRP	A	383	7.836	-8.138		1,00	20.41
		MOTA	616	CE3	TRP	A	383	9.012	-6.420		1.00	22.06
	55											
	33	MOTA	617	CD1	TRP	A	383	9.506	-9.189		1.00	23.38
		MOTA	618	NE1	TRP	A	383	8.142	-9.159		1.00	22.59
		MOTA	619	CZ2	TRP	A	383	6.598	-7.713		1.00	21.98
		MOTA	620	CZ3	TRP	A	383	7.780	-5.998	-3.968	1.00	25.50
		MOTA	621	CH2	TRP	A	383	6.589	-6.647	-3.587	1.00	23.11
	60	ATOM	622	С	TRP	A	383	11.448	-5.564		1.00	19.18
		MOTA	623	0	TRP	A	383	11.972	-4.663		1.00	19.27
		ATOM	624	N	LEU	A	384	10.273	-5.396		1.00	18.32
		AION	027	Τ.	الاند	7	JU4	10.273	5.576	0.507	1.00	10.02

	5	MOTA	741	CA	HIS	A	398	13.342		1.00	39.21
		ATOM	742	CB	HIS	Α	398	12.924		1.00	39.05
		MOTA	743	CG	HIS	Α	398	13.870		1.00	41.57
		ATOM	744	CD2	HIS	Α	398	13.904		1.00	39.28
		MOTA	745	ND1	HIS	Α	398	14.940		1.00	41.85
	10	ATOM	746	CE1	HIS	A	398	15.592	14.086 -9.220	1.00	40.88
		ATOM	747	NE2	HIS	A	398	14.985	14.969 -9.993	1.00	42.30
		ATOM	748	С	HIS	A	398	12.216	16.332 -4.944	1.00	37.04
		ATOM	749	0	HIS	A	398	11.282	15.535 -4.864	1.00	36.51
		MOTA	750	N	PRO	A	399	12.283	17.427 -4.171	1.00	39.19
	15	ATOM	751	CD	PRO	Α	399	13.328	18.467 -4.198	1.00	35.36
	15	ATOM	752	CA	PRO	A	399	11.243	17.709 -3.173	1.00	37.10
		ATOM	753	CB	PRO	A	399	11.603	19.101 -2.654	1.00	37.86
		MOTA	754	CG	PRO	A	399	13.050	19.267 -2.963	1.00	35.83
		ATOM	75 5	C	PRO	A	399	9.828	17.663 -3.744	1.00	37.02
	20		756	0	PRO	A	399	9.554	18.249 -4.789	1.00	38.52
	20	MOTA		N	GLY	A	400	8.938	16.954 -3.057	1.00	33.58
		ATOM	757	CA	GLY	A	400	7.559	16.865 -3.503	1.00	32.12
		ATOM	758 750	C	GLY	A	400	7.230	15.706 -4.428	1.00	32.43
		MOTA	759	0	GLY	A	400	6.063	15.344 -4.574	1.00	33.21
1 57555	25	ATOM	760		LYS	A	401	8.237	15.112 -5.055	1.00	31.35
	25	ATOM	761	N		A	401	7.972	14.007 -5.966	1.00	30.75
111		MOTA	762	CA	LYS	A	401	8.235	14.430 -7.415	1.00	35.43
IJ		ATOM	763	CB	LYS		401	8.130	15.927 -7.675	1.00	35.15
IJ		MOTA	764	CG	LYS	A	401	9.096	16.353 -8.774	1.00	36.88
į.	20	MOTA	765	CD	LYS	A	401	8.733	17.721 -9.331	1.00	36.71
1.4	30	MOTA	766	CE	LYS	A A	401	7.295	18.027 -9.116	1.00	34.22
		MOTA	767	NZ C	LYS LYS	A	401	8.768	12.746 -5.677	1.00	30.97
		ATOM	768 769	0	LYS	A	401	9.809	12.776 -5.006	1.00	27.60
#: 		ATOM ATOM	770	N	LEU	A	402	8.256	11.635 -6.197	1.00	27.28
	35	ATOM	771	CA	LEU	A	402	8.889	10.334 -6.050	1.00	29.07
144	55	ATOM	772	CB	LEU	A	402	7.866	9.294 -5.590	1.00	22.55
113		ATOM	773	CG	LEU	A	402	7.265	9.555 -4.207	1.00	24.94
		ATOM	774	CD1	LEU	A	402	6.126	8.583 -3.937	1.00	19.32
123		MOTA	775	CD2	LEU	A	402	8.355	9.416 -3.157	1.00	21.54
il.	40	MOTA	776	C	LEU	А	402	9.448	9.948 -7.414	1.00	28.78
	10	ATOM	777	0	LEU	Α	402	8.704	9.836 -8.389	1.00	29.98
		ATOM	778	N	LEU	A	403	10.761	9.770 -7.487	1.00	27.57
		MOTA	779	CA	LEU	A	403	11.393	9.400 -8.744	1.00	27.17
		ATOM	780	CB	LEU	Α	403	12.825	9.937 -8.816	1.00	26.95
	45	ATOM	781	CG	LEU	A	403	13.401	10.027-10.238	1.00	30.42
		ATOM	782	CD1	LEU	Α	403	14.519	11.046-10.288	1.00	30.76
		ATOM	783	CD2	LEU	A	403	13.915	8.665-10.676	1.00	33.11
		ATOM	784	C	LEU	Α	403	11.419	7.891 -8.901	1.00	24.78
		ATOM	785	0	LEU	A	403	12.428	7.257 -8.619	1.00	24.68
	50	ATOM	786	N	PHE	Α	404	10.306	7.319 -9.344	1.00	23.11
		ATOM	787	CA	PHE	A	404	10.239	5.881 -9.546	1.00	26.93
		MOTA	788	CB	PHE	Α	404	8.826	5.470 -9.946	1.00	27.04
		MOTA	789	CG	PHE	A	404	7.850	5.513 -8.816	1.00	27.89
		ATOM	790	CD1	PHE	A	404	7.028	6.623 -8.631	1.00	26.20
	55	MOTA	791	CD2	PHE	A	404	7.750	4.444 -7.925	1.00	23.10
		ATOM	792	CE1	PHE	A	404	6.116	6.668 -7.573	1.00	25.29
		ATOM	793	CE2	PHE	Α	404	6.845	4.481 -6.870	1.00	21.01
		MOTA	794	CZ	PHE	Α	404	6.026	5.595 -6.693	1.00	22.91
		ATOM	795	С	PHE	A	404	11.232	5.507-10.637	1.00	26.04
	60	MOTA	796	0	PHE	Α	404	11.882	4.464-10.578	1.00	27.27
		ATOM	797	N	ALA	A	405	11.348	6.383-11.626	1.00	28.80
		ATOM	798	CA	ALA	Α	405	12.271	6.195-12.740	1.00	29.21
								1.62			

	5	ATOM	1147	С	LYS	Α	449	20.629	-2.436	2.548	1.00	20.33
		MOTA	1148	0	LYS	Α	449	19.800	-3.345	2.552	1.00	20.57
		MOTA	1149	N	SER	A	450	21.924	-2.637	2.777	1.00	19.25
		ATOM	1150	CA	SER	A	450	22.451	-3.965	3.074	1.00	21.84
		MOTA	1151	CB	SER	Α	450	23.982	-3.953	3.041	1.00	20.59
	10	MOTA	1152	OG	SER	Α	450	24.460	-3.975	1.702	1.00	29.78
		MOTA	1153	С	SER	Α	450	21.975	-4.408	4.454	1.00	21.58
		MOTA	1154	0	SER	A	450	21.728	-5.590	4.682	1.00	20.06
		MOTA	1155	N	ILE	Α	451	21.853	-3.449	5.369	1.00	22.20
		MOTA	1156	CA	ILE	Α	451	21.385	-3.741	6.726	1.00	22.82
	15	MOTA	1157	CB	ILE	A	451	21.452	-2.476	7.616	1.00	19.62
		ATOM	1158	CG2	ILE	Α	451	20.593	-2.658	8.886	1.00	21.11
		ATOM	1159	CG1	ILE	A	451	22.909	-2.210	7.999	1.00	22.20
		MOTA	1160	CD1	ILE	A	451	23.115	-0.960	8.850	1.00	24.48
		MOTA	1161	C	ILE	A	451	19.952	-4.250	6.662	1.00	21.82
	20	MOTA	1162	0	ILE	A	451	19.575	-5.184	7.369	1.00	21.72
		MOTA	1163	N	$_{ m ILE}$	Α	452	19.152	-3.642	5.795	1.00	20.18
		MOTA	1164	CA	ILE	A	452	17.763	-4.058	5.649	1.00	18.13
		MOTA	1165	CB	ILE	Α	452	17.024	-3.145	4.627	1.00	19.72
		ATOM	1166	CG2	ILE	А	452	15.720	-3.792	4.169	1.00	18.99
122	25	ATOM	1167	CG1	$_{ m ILE}$	A	452	16.725	-1.788	5.282	1.00	18.33
		MOTA	1168	CD1	ILE	A	452	16.284	-0.707	4.306	1.00	23.25
222 2		MOTA	1169	C	ILE	A	452	17.725	-5.517	5.191	1.00	19.50
111		MOTA	1170	0	ILE	A	452	16.980	-6.340	5.737	1.00	17.60
£.4		MOTA	1171	N	LEU	A	453	18.555	-5.844	4.209	1.00	19.23
	30	MOTA	1172	CA	LEU	Α	453	18.589	-7.205	3.679	1.00	21.60
		MOTA	1173	CB	LEU	A	453	19.624	-7.316	2.554	1.00	21.50
		MOTA	1174	CG	LEU	A	453	19.835	-8.729	1.989	1.00	25.06
 Ei		MOTA	1175	CD1	LEU	A	453	18.550	-9.250	1.364	1.00	25.27
		MOTA	1176	CD2	LEU	A	453	20.948	-8.694	0.953	1.00	24.73
H	35	MOTA	1177	С	LEU	A	453	18.906	-8.245	4.746	1.00	19.41
L		MOTA	1178	0	LEU	A	453	18.198	-9.241	4.891	1.00	20.75
1		MOTA	1179	N	LEU	A	454	19.966	-7.997	5.499	1.00	21.35
		MOTA	1180	CA	LEU	A	454	20.410	-8.925	6.530	1.00	23.67
		MOTA	1181	CB	LEU	A	454	21.870	-8.625	6.878	1.00	20.69
.22	40	MOTA	1182	CG	LEU	A	454	22.816	-8.584	5.673	1.00	24.92
		MOTA	1183	CD1	LEU	A	454	24.222	-8.268	6.132	1.00	24.27 22.84
		MOTA	1184	CD2	LEU	A	454	22.785	-9.913	4.952		
		MOTA	1185	С	LEU	A	454	19.572	-8.945	7.807	1.00	26.06 27.44
		MOTA	1186	0	LEU	A	454	19.413	-9.997	8.438	1.00	
	45	MOTA	1187	N	ASN	A	455	19.011	-7.795	8.167	1.00	25.01 26.10
		MOTA	1188	CA	ASN	A	455	18.240	-7.681	9.400		22.67
		MOTA	1189	CB	ASN	A	455	18.439	-6.295		1.00	26.67
		MOTA	1190	CG	ASN	A	455	17.627	-6.109			25.16
		MOTA	1191	OD1	ASN	A	455	17.899	-6.751		1.00	
	50	MOTA	1192	ND2	ASN	A	455	16.615		11.212	1.00	20.73 25.78
		MOTA	1193	C	ASN	A	455	16.739	-7.957		1.00	
		MOTA	1194	0	ASN	A	455	16.230		10.380	1.00	29.22 28.51
		MOTA	1195	N	SER	A	456	16.027	-7.549	8.381	1.00	32.52
		MOTA	1196	CA	SER	A	456	14.578	-7.704	8.371	1.00	35.98
	55	MOTA	1197	CB	SER	A	456	14.019	-7.213	7.033	1.00	
		MOTA	1198	OG	SER	A	456	14.266	-5.818	6.897	1.00	30.88
		MOTA	1199	С	SER	A	456	14.033	-9.086	8.711	1.00	33.00
		MOTA	1200	0	SER	A -	456	13.112	-9.202	9.523	1.00	33.07
	<i>~</i> ^	MOTA	1201		GLY	A	457	14.597		8.117	1.00	28.40
	60	MOTA	1202	CA	GLY	A	457		-11.464	8.413	1.00 1.00	36.28 40.41
		MOTA	1203		GLY	A	457		-12.289	9.277		38.20
		ATOM	1204	0	GLY	A	457		-13.486	9.456	1.00	30.∠∪
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58.56

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MOTA

	5	ATOM	2597	C	MET	В	388	6.004	-5.332 23.133	1.00	20.19
		MOTA	2598	0	MET	В	388	6.460	-6.366 23.636	1.00	21.50
		ATOM	2599	N	ILE	В	389	6.707	-4.203 23.074	1.00	15.34
		ATOM	2600	CA	ILE	В	389	8.044	-4.209 23.634	1.00	15.59
		ATOM	2601	CB	ILE	В	389	8.836	-2.911 23.322	1.00	14.95
	10	MOTA	2602	CG2	ILE	В	389	8.330	-1.746 24.158	1.00	12.81
		MOTA	2603	CG1	ILE	В	389	10.325	-3.164 23.602	1.00	17.24
		MOTA	2604	CD1	ILE	В	389	11.228	-1.972 23.357	1.00	15.65
		ATOM	2605	C	ILE	В	389	7.950	-4.446 25.147	1.00	14.30
		ATOM	2606	0	ILE	В	389	8.844	-5.044 25.739	1.00	18.72
	15	ATOM	2607	N	GLY	В	390	6.855	-4.007 25.761	1.00	13.99
		ATOM	2608	CA	GLY	В	390	6.681	-4.219 27.189	1.00	14.87
		ATOM	2609	C	GLY	В	390	6.444	-5.702 27.463	1.00	18.54
		ATOM	2610	0	GLY	В	390	6.989	-6.282 28.403	1.00	16.54
		ATOM	2611	N	LEU	В	391	5.623	-6.325 26.628	1.00	16.15
	20	ATOM	2612	CA	LEU	В	391	5.334	-7.743 26.775	1.00	18.91
		ATOM	2613	СВ	LEU	В	391	4.332	-8.179 25.699	1.00	19.55
		ATOM	2614	CG	LEU	В	391	4.157	-9.689 25.457	1.00	20.91
		ATOM	2615	CD1	LEU	В	391	3.580	-10.351 26.699	1.00	19.41
		ATOM	2616	CD2	LEU	В	391	3.232	-9.913 24.268	1.00	20.70
	25	ATOM	2617	C	LEU	В	391	6.649	-8.518 26.625	1.00	20.31
13	23	ATOM	2618	0	LEU	B	391	7.002	-9.352 27.465	1.00	18.66
30		ATOM	2619	N	VAL	В	392	7.378	-8.215 25.557	1.00	18.71
111		ATOM	2620	CA	VAL	В	392	8.649	-8.868 25.278	1.00	19.51
Ħ.		ATOM	2621	CB	VAL	В	392	9.288	-8.281 24.005	1.00	23.77
7.5	30	ATOM	2622	CG1	VAL	В	392	10.751	-8.687 23.920	1.00	24.63
194	50	MOTA	2623	CG2	VAL	В	392	8.520	-8.773 22.767	1.00	19.94
<u></u>		ATOM	2624	C	VAL	В	392	9.615	-8.707 26.450	1.00	22.80
		ATOM	2625	0	VAL	В	392	10.336	-9.637 26.811	1.00	19.36
##		ATOM	2626	N	TRP	В	393	9.617	-7.522 27.046	1.00	22.10
	35	ATOM	2627	CA	TRP	В	393	10.492	-7.241 28.171	1.00	23.20
IJ	33	ATOM	2628	CB	TRP	В	393	10.388	-5.773 28.578	1.00	19.22
IJ		ATOM	2629	CG	TRP	В	393	11.056	-5.479 29.895	1.00	22.53
			2630	CD2	TRP	В	393	12.453	-5.591 30.193	1.00	20.36
		ATOM ATOM	2631	CE2	TRP	В	393	12.624	-5.208 31.545	1.00	25.65
1	40		2632	CE2	TRP	В	393	13.578	-5.976 29.449	1.00	22.12
	40	ATOM		CD1	TRP	В	393	10.452	-5.046 31.044	1.00	23.02
		ATOM	2633		TRP	В	393	11.387	-4.881 32.037	1.00	24.91
		ATOM	2634	NE1	TRP	В	393	13.876	-5.200 32.171	1.00	23.00
		MOTA	2635	CZ2		В	393	14.829	-5.968 30.072	1.00	23.98
	15	MOTA	2636	CZ3	${ t TRP}$	В	393	14.964	-5.582 31.423	1.00	23.20
	45	ATOM	2637	CH2				10.208	-8.114 29.388	1.00	24.36
		ATOM	2638	C	TRP	В	393 393	11.128	-8.717 29.944	1.00	23.04
		ATOM	2639	0	TRP	В		8.952	-8.189 29.819	1.00	21.29
		ATOM	2640	N	ARG	В	394		-9.003 30.990	1.00	22.43
	50	ATOM	2641	CA	ARG	В	394	8.680	-8.601 31.667	1.00	23.97
	50	ATOM	2642	CB	ARG	В	394	7.365	-8.149 30.759	1.00	26.16
		ATOM	2643	CG	ARG	В	394	6.259			20.86
		MOTA	2644	CD	ARG	В	394	5.026	-7.727 31.574	1.00	
		ATOM	2645	NE	ARG	В	394	3.817	-7.937 30.786	1.00	19.54
		MOTA	2646	CZ	ARG	В	394	3.327	-7.059 29.915	1.00	20.58
	55	MOTA	2647	NH1	ARG	В	394	3.944	-5.902 29.722	1.00	17.41
		ATOM	2648	NH2	ARG	В	394	2.229	-7.347 29.220	1.00	16.82
		MOTA	2649	C	ARG	В	394		-10.502 30.713	1.00	21.78
		ATOM	2650	0	ARG	В	394		-11.294 31.648	1.00	23.44
		MOTA	2651	N	SER	В	395		-10.880 29.438	1.00	17.10
	60	MOTA	2652	CA	SER	В	395		-12.289 29.041	1.00	25.08
		MOTA	2653	CB	SER	В	395		-12.473 27.638	1.00	19.47
		ATOM	2654	OG	SER	В	395	6.832	-12.136 27.619	1.00	21.73
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	5	MOTA	3003	CE	MET	В	438	21.693	-5.657 21.943	1.00	35.91
		MOTA	3004	С	MET	В	438	21.877	-9.122 25.738	1.00	28.81
		MOTA	3005	0	MET	В	438	22.686	-8.240 26.013	1.00	30.13
		ATOM	3006	N	ASN	В	439	21.120	-9.721 26.646	1.00	27.14
		MOTA	3007	CA	ASN	В	439	21.199	-9.359 28.038	1.00	27.34
	10	MOTA	3008	CB	ASN	В	439	22.592	-9.524 28.598	1.00	34.85
		MOTA	3009	CG	ASN	В	439	22.624	-9.480 30.080	1.00	38.58
		ATOM	3010	OD1	ASN	В	439	21.584	-9.620 30.724	1.00	42.99
		ATOM	3011	ND2	ASN	В	439	23.801	-9.260 30.666	1.00	41.14
		ATOM	3012	С	ASN	В	439	20.745	-7.903 28.212	1.00	26.24
	15	ATOM	3013	0	ASN	В	439	21.396	-7.106 28.891	1.00	19.76
		ATOM	3014	N	LEU	В	440	19.625	-7.564 27.573	1.00	24.90
		ATOM	3015	CA	LEU	В	440	19.061	-6.214 27.633	1.00	25.04
		ATOM	3016	CB	LEU	В	440	17.761	-6.157 26.818	1.00	22.36
		ATOM	3017	CG	LEU	В	440	17.087	-4.786 26.740	1.00	26.33
	20	ATOM	3018	CD1	LEU	В	440	17.958	-3.843 25.923	1.00	28.33
		ATOM	3019	CD2	LEU	В	440	15.704	-4.914 26.111	1.00	24.81
		ATOM	3020	C	LEU	В	440	18.782	-5.785 29.074	1.00	24.71
		ATOM	3021	0	LEU	В	440	18.131	-6.504 29.830	1.00	26.96
		ATOM	3022	N	GLN	В	441	19.268	-4.609 29.452	1.00	25.54
, major	25	ATOM	3023	CA	GLN	В	441	19.060	-4.099 30.807	1.00	25.82
	23	ATOM	3024	CB	GLN	В	441	20.250	-3.231 31.234	1.00	30.41
		ATOM	3025	CG	GLN	В	441	21.572	-3.956 31.228	1.00	30.50
		ATOM	3026	CD	GLN	В	441	21.610	-5.028 32.279	1.00	32.75
IJ		ATOM	3027	OE1	GLN	В	441	21.539	-4.772 33.473	1.00	36.52
1.4	30	ATOM	3027	NE2	GLN	В	441	21.703	-6.288 31.823	1.00	31.09
`*.j	30	ATOM	3029	C	GLN	В	441	17.789	-3.265 30.883	1.00	26.93
14 14]		ATOM	3030	0	GLN	В	441	17.303	-2.768 29.866	1.00	25.40
**		ATOM	3031	N	GLY	В	442	17.266	-3.105 32.096	1.00	24.56
31		ATOM	3032	CA	GLY	В	442	16.058	-2.327 32.293	1.00	22.82
	35	ATOM	3032	C	GLY	В	442	16.217	-0.873 31.885	1.00	24.19
W	33	ATOM	3034	0	GLY	В	442	15.290	-0.279 31.341	1.00	20.21
W		ATOM	3035	N	GLU	В	443	17.387	-0.293 32.141	1.00	22.92
	•	ATOM	3036	CA	GLU	В	443	17.635	1.102 31.778	1.00	23.33
		MOTA	3037	CB	GLU	В	443	18.960	1.590 32.378	1.00	24.26
ũ	40	ATOM	3038	CG	GLU	В	443	19.005	1.525 33.895	1.00	32.31
	-10	ATOM	3039	CD	GLU	В	443	19.701	0.270 34.402	1.00	37.68
		ATOM	3040	OE1	GLU	В	443	19.343	-0.841 33.948	1.00	35.23
		ATOM	3041	OE2	GLU	В	443	20.607	0.394 35.252	1.00	42.47
		MOTA	3042	C	GLU	В	443	17.662	1.278 30.262	1.00	23.08
	45	MOTA	3043	0	GLU	В	443	17.265	2.328 29.747	1.00	21.80
	73	ATOM	3044	И	GLU	В	444	18.128	0.253 29.552	1.00	21.16
		ATOM	3045	CA	GLU	В	444	18.182	0.302 28.093	1.00	22.60
		ATOM	3045	CB	GLU	В	444	19.046	-0.834 27.545	1.00	20.89
			3047	CG	GLU	В	444	20.545	-0.617 27.705	1.00	23.24
	50	ATOM	3047	CD	GLU	В	444	21.340	-1.869 27.393	1.00	22.11
	50	ATOM	3049	OE1	GLU	В	444	20.817	-2.978 27.629	1.00	20.89
		ATOM	3050	OE2	GLU	В	444	22.488	-1.746 26.914	1.00	25.49
		MOTA		C	GLU	В	444	16.758	0.155 27.552	1.00	21.06
		MOTA	3051		GLU	В	444	16.737	0.822 26.597	1.00	23.73
	55	ATOM	3052	O N			444	15.987	-0.730 28.176	1.00	19.01
	55	ATOM	3053	N Ca	PHE PHE	В	445	14.600	-0.969 27.792	1.00	19.44
		ATOM	3054	CA		В			-2.067 28.675	1.00	18.12
		MOTA	3055	CB	PHE	В	445	13.989	-2.055 28.709	1.00	18.13
		ATOM	3056	CG	PHE	В	445	12.483		1.00	18.34
	60	ATOM	3057	CD1	PHE	В	445	11.746	-2.386 27.575 -1.694 29.872		16.59
	60	ATOM	3058	CD2	PHE	В	445	11.802		1.00	17.15
		ATOM	3059	CE1	PHE	В	445	10.346	-2.359 27.592	1.00	
		ATOM	3060	CE2	PHE	В	445	10.406	-1.662 29.903	1.00	21.99

_	3 moss	2261 0	7 DIII	-	445	9.674	-1.997 28.755	1.00	16.01
5	ATOM	3061 C		В	445			1.00	15.87
	MOTA	3062 C	PHE	В	445	13.758	0.304 27.888		
	MOTA	3063 O	PHE	В	445	13.008	0.617 26.966	1.00	20.27
	MOTA	3064 N	VAL	В	446	13.872	1.044 28.986	1.00	15.90
	MOTA	3065 C	A VAL	В	446	13.074	2.269 29.112	1.00	16.78
10	MOTA	3066 C	B VAL	В	446	13.165	2.895 30.531	1.00	18.32
	ATOM	3067 C	G1 VAL	В	446	12.574	1.923 31.551	1.00	21.14
	MOTA		G2 VAL	В	446	14.598	3.251 30.879	1.00	21.04
	ATOM	3069 C		В	446	13.450	3.295 28.051	1.00	17.91
	ATOM	3070 0		В	446	12.596	4.028 27.561	1.00	19.37
15	ATOM	3071 N		В	447	14.723	3.335 27.674	1.00	18.81
13	ATOM	3071 N		В	447	15.161	4.255 26.635	1.00	17.34
				В	447	16.682	4.224 26.512	1.00	19.33
	ATOM					17.538	5.134 27.798	1.00	23.60
	ATOM	3074 S		В	447		3.826 25.301	1.00	18.09
20	MOTA	3075 C		В	447	14.537			17.52
20	MOTA	3076 O		В	447	13.988	4.643 24.563	1.00	
	ATOM	3077 N		В	448	14.623	2.533 25.006	1.00	15.60
	MOTA	3078 C		В	448	14.072	1.994 23.767	1.00	16.67
	ATOM	3079 C		В	448	14.328	0.490 23.684	1.00	14.82
	MOTA	3080 C	G LEU	В	448	15.730	0.009 23.301	1.00	23.57
25	ATOM	3081 C	D1 LEU	В	448	15.722	-1.522 23.169	1.00	21.61
1, 120,00 1, 120,00 1, 120,00	MOTA	3082 C	D2 LEU	В	448	16.167	0.658 21.986	1.00	18.92
	ATOM	3083 C	LEU	В	448	12.573	2.249 23.652	1.00	15.98
222	ATOM	3084 0		В	448	12.078	2.633 22.590	1.00	18.91
113	ATOM	3085 N		В	449	11.849	2.037 24.745	1.00	17.94
30	MOTA	3086 C		В	449	10.405	2.232 24.733	1.00	16.66
	MOTA	3087 C		В	449	9.796	1.745 26.047	1.00	16.45
	ATOM	3088 C		В	449	8.285	1.861 26.115	1.00	16.12
	ATOM	3089 C		В	449	7.730	0.952 27.193	1.00	19.09
Ħ					449	8.201	1.380 28.580	1.00	17.04
35	ATOM	3090 C		В		7.159	1.088 29.593	1.00	17.25
35	MOTA	3091 N		В	449		3.696 24.486	1.00	18.78
l.i	MOTA	3092 C		В	449	10.058			
	MOTA	3093 0		В	449	9.103	3.996 23.769	1.00	14.84
. 27	MOTA	3094 N		В	450	10.837	4.610 25.059	1.00	14.50
11 12 14 40	MOTA		A SER	В	450	10.591	6.032 24.849	1.00	17.11
40	MOTA		B SER	В	450	11.440	6.866 25.815	1.00	21.20
	MOTA	3097 O	G SER	В	450	10.859	6.868 27.108	1.00	30.66
	ATOM	3098 C	SER	В	450	10.921	6.418 23.405	1.00	17.84
	MOTA	3099 O	SER	В	450	10.279	7.292 22.821	1.00	18.82
	ATOM	3100 N	ILE	В	451	11.926	5.768 22.828	1.00	16.88
45	MOTA	3101 C	:A ILE	В	451	12.305	6.063 21.450	1.00	17.11
	MOTA		B ILE	В	451	13.564	5.268 21.025	1.00	16.69
	ATOM		G2 ILE	В	451	13.724	5.298 19.505	1.00	19.31
	ATOM		G1 ILE	В	451	14.804	5.897 21.676	1.00	18.96
	ATOM		D1 ILE	В	451	16.083	5.130 21.431	1.00	18.98
50	ATOM	3105 C		В	451	11.142	5.711 20.527	1.00	18.09
30				В	451	10.820	6.464 19.608	1.00	17.07
	ATOM	3107 C		В	452	10.505	4.571 20.786	1.00	18.13
	ATOM	3108 N				9.373	4.137 19.976	1.00	16.77
	MOTA		CA ILE	В	452			1.00	17.40
	MOTA		B ILE	В	452	8.804	2.775 20.477		
55	ATOM		G2 ILE	В	452	7.464	2.496 19.831	1.00	14.33
	MOTA		CG1 ILE	В	452	9.763	1.635 20.107	1.00	15.36
	MOTA	3113 C	CD1 ILE	В	452	9.449	0.323 20.805	1.00	17.76
	ATOM	3114 0		В	452	8.271	5.195 20.024	1.00	17.47
	ATOM	3115 C) ILE	В	452	7.733	5.586 18.992	1.00	16.50
60	ATOM	3116 N	I LEU	В	453	7.943	5.665 21.222	1.00	16.06
	ATOM		CA LEU	В	453	6.903	6.680 21.374	1.00	17.17
	ATOM		CB LEU	В	453	6.736	7.061 22.850	1.00	16.23
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	5	HETATM	4105	0	НОН	73	6.291	14.878 29.070	1.00	28.21
		HETATM	4106	0	HOH	74	-1.721	6.480 13.381	1.00	49.91
		HETATM	4107	0	НОН	75		-15.427 26.194	1.00	24.17
		HETATM	4108	0	HOH	76	5.029	7.461 17.718	1.00	18.91
		HETATM	4109	0	HOH	77	3.758	2.086 14.306	1.00	28.28
	10	HETATM	4110	0	HOH	78	-1.390	-18.739 33.183	1.00	41.11
		HETATM	4111	0	HOH	79	12.703	-8.687 32.119	1.00	36.21
		HETATM	4112	0	НОН	80	22.270	-6.451 14.844	1.00	33.21
		HETATM	4113	0	НОН	81	1.458	4.605 34.026	1.00	23.59
		HETATM	4114	0	НОН	82	1.759	-2.158 30.374	1.00	28.78
	15	HETATM	4115	0	НОН	83		-21.372 23.188	1.00	31.14
		HETATM	4116	0	НОН	84	36.525	0.463 20.792	1.00	45.26
		HETATM	4117	ō	НОН	85	13.832	9.696 13.792	1.00	33.12
		HETATM	4118	Ō	НОН	86	31.166	6.635 24.924	1.00	35.19
		HETATM	4119	Ö	НОН	87		-10.389 34.180	1.00	48.80
	20	HETATM	4120	Õ	НОН	88	9.581	-6.956 34.136	1.00	42.95
		HETATM	4121	0	нон	89	-1.563	15.887 27.596	1.00	39.35
		HETATM	4122	Ö	НОН	90	-5.286	10.345 32.757	1.00	35.20
		HETATM	4123	0	нон	91	15.035	0.607 13.339	1.00	29.53
		HETATM	4124	0	нон	92	-10.984	-1.500 30.272	1.00	29.84
	25	HETATM	4125	0	нон	93	-7.239	-0.271 -1.207		
and,	43	HETATM	4125	0	HOH	93 94	18.022	-4.902 34.286	1.00	48.98
		HETATM	4127	0	HOH	95	29.347	-6.319 19.920	1.00	35.28
ngq.		HETATM	4128	0	нон	96		-19.369 20.945	1.00	37.20
j		HETATM	4129	0	HOH	97				30.23
ž	30	HETATM	4130	0	HOH	97 98	31.496	4.614 18.716	1.00	38.79
3	30	HETATM	4131	0	HOH	96 99	26.567	9.759 25.629	1.00	29.72
=		HETATM	4131	0	HOH		2.848	14.531 1.134	1.00	38.08
		HETATM				100	-9.373	5.699 -7.953	1.00	53.23
		HETATM	4133 4134	0	НОН НОН	101	-10.137	-0.553 -6.742	1.00	47.72
1	35					102	10.558	-10.363 15.403	1.00	40.97
E C	33	HETATM HETATM	4135 4136	0	HOH	103	21.079	17.166 18.929	1.00	32.40
		HETATM		0	HOH	104	25.810	-5.921 22.506	1.00	37.69
		HETATM	4137 4138	0	НОН НОН	105	22.493	-1.311 34.465	1.00	49.94
		HETATM	4139	0		106	19.317	10.977 38.703	1.00	40.60
i	40	HETATM	4140	0	HOH	107	4.479 20.418	13.951 3.045	1.00	45.33
	TU	HETATM	4140	0	НОН НОН	108 109		19.353 34.044	1.00	42.18
		HETATM	4141		HOH		-3.065	8.936 14.062	1.00	38.41
		HETATM	4142		HOH	110	26.856	-4.674-10.940 -6.387 5.614	1.00	55.67
		HETATM	4144		HOH	111 112	2.032		1.00	42.23
	45	HETATM	4145		HOH		0.601	0.228-17.268 13.488-14.050	1.00	40.57
	73	HETATM		0	HOH	113	4.903 3.986		1.00	47.72
		HETATM		0		114		16.140 -0.960	1.00	40.66
		HETATM		0	НОН НОН	115	12.968 7.170	-19.561 2.741	1.00	40.76
		HETATM	4149		HOH	116	-1.966	15.583 2.599	1.00	43.69
	50	HETATM	4149		HOH	117	29.030	10.606 3.572 10.644 6.707	1.00	52.63
	50					118				42.54
		HETATM	4151		HOH	119		4.354 8.374	1.00	38.69
		HETATM	4152		НОН	120	29.086	17.119 19.272	1.00	45.51
		HETATM	4153		НОН	121	24.614	17.609 20.174	1.00	53.55
	55	HETATM	4154		HOH	122	-15.318	0.362 26.686	1.00	36.77
	JJ	HETATM	4155		HOH	123		-24.786 28.325	1.00	39.64
		HETATM		0	НОН	124	21.728	22.178 31.983	1.00	43.73
		HETATM	4157		HOH	125	31.650	-7.370 21.642	1.00	40.53
		HETATM		0	НОН	126	25.421	10.436 21.161	1.00	32.31
	60	HETATM	4159		HOH	127	10.317	-9.457 12.998	1.00	37.77
	60	HETATM		0	НОН	128	22.723	14.887 15.427	1.00	47.90
		HETATM		0	HOH	129		9.556 37.596	1.00	47.81
		HETATM	4162	U	HOH	130	27.987	13.557 7.167	1.00	41.15

5	HETATM	4163	0	нон	101	20 700	16 400 7 500	1 00	E0 4E
,			_		131	30.798	16.499 7.588	1.00	58.47
	HETATM	4164	0	HOH	132	10.071	-0.571-20.393	1.00	38.79
	HETATM	4165	0	HOH	133	9.562	8.334-21.392	1.00	36.80
	HETATM	4166	0	HOH	134	6.712	6.058 8.822	1.00	37.43
	HETATM	4167	0	HOH	135	5.927	8.454 10.594	1.00	42.34
10	HETATM	4168	0	HOH	136	4.472	6.306 10.973	1.00	37.35
	HETATM	4169	0	HOH	137	6.792	7.721 7.051	1.00	47.23
	HETATM	4170	0	HOH	138	24.513	11.582 33.724	1.00	45.55
	HETATM	4171	0	HOH	139	-2.528	-20.361 12.354	1.00	52.13
	HETATM	4172	0	HOH	140	-7.864	7.706 19.248	1.00	47.82
15	HETATM	4173	0	HOH	141	11.577	-16.962 24.398	1.00	39.43
	HETATM	4174	0	HOH	142	18.087	12.263 -5.507	1.00	33.36
	HETATM	4175	0	HOH	143	-6.816	-14.190 10.674	1.00	51.32
	HETATM	4176	0	HOH	144	-7.377	-16.701 33.528	1.00	57.11
	HETATM	4177	0	HOH	145	-5.379	-20.107 32.689	1.00	43.01
20	HETATM	4178	0	HOH	146	8.766	-7.947-16.274	1.00	49.96
	HETATM END	4179	0	HOH	147	10.946	-7.937-18.142	1.00	55.67
	EMD								

Appendix 3

Atomic Coordinates for Human ER α Complexed With OHT

	10	CRYST1	58.2	42 58.	242	277.46	7	90.00	90.00	120.00	F	65	2	2	12
	10	ORIGX1	7 /	00000	0.00	00000	0	.000000	0.0	0000					
		ORIGX2		000000		00000		.000000		0000 0000					
								.000000							
		ORIGX3		000000		00000				0000					
	15	SCALE1		017170		09913		.000000		0000					
	15	SCALE2		000000		19826		.000000		0000					
		SCALE3	0.0	000000	0.00	00000	Ü	.003604	0.0	0000					
		ATOM	1	CB	LEU	306		6.638	11.50			1.00		1.2	
		ATOM	2	С	LEU	306		7.381	10.684			1.00		1.4	
	20	ATOM	3	0	LEU	306		6.407	11.02			1.00		2.0	
		ATOM	4	N	LEU	306		6.369	9.12			1.00		2.3	
		ATOM	5	CA	LEU	306		7.232	10.33			1.00		1.3	
		ATOM	6	N	ALA	307		8.609	10.60			1.00		0.5	
		ATOM	7	CA	ALA	307		8.891	10.91			1.00		8.7	
	25	ATOM	8	CB	ALA	307	-	10.318	10.50			1.00		9.7	
Ð.		ATOM	9	С	ALA	307		8.692	12.393	8.42	9	1.00		7.5	
IJ		ATOM	10	0	ALA	307		8.451	12.77			1.00		7.6	
ij		ATOM	11	N	LEU	308		8.789	13.228			1.00		5.8	
įż	• •	ATOM	12	CA	LEU	308		8.638	14.668			1.00		5.6	
1.4	30	ATOM	13	CB	LEU	308		9.298	15.402			1.00		7.4	
		ATOM	14	CG	LEU	308		10.637	14.822			1.00		9.1	
'n.		ATOM	15	CD1	LEU	308		10.474	14.189			1.00		0.3	
51		ATOM	16	CD2	LEU	308	-	11.694	15.92			1.00		3.4	
	2.5	ATOM	17	C	LEU	308		7.190	15.13			1.00		5.5	
laj	35	ATOM	18	0	LEU	308		6.935	16.30			1.00		5.5	
		ATOM	19	N	SER	309		6.246	14.208			1.00		7.0	
		ATOM	20	CA	SER	309		4.828	14.54			1.00		5.4	
ı.		MOTA	21	CB	SER	309		4.034	13.89			1.00		5.7	
:::::	40	ATOM	22	OG -	SER	309		4.071	12.479			1.00		7.2	
	40	ATOM	23	C	SER	309		4.261	14.09			1.00		5.1	
		ATOM	24	0	SER	309		3.166	14.50			1.00		5.1	
		ATOM	25	N	LEU	310		5.016	13.25			1.00		1.3	
		ATOM	26	CA	LEU	310		4.591	12.749			1.00		3.5	
	15	ATOM	27	CB	LEU	310		5.651	11.81			1.00		1.4	
	45	ATOM	28	CG	LEU	310		5.586	10.333			1.00		5.4	
		ATOM	29	CD1	LEU	310		5.530	10.200			1.00		7.0	
		MOTA	30	CD2	LEU	310		6.809	9.610			1.00		7.2	
		MOTA	31	С О	LEU	310		4.330	13.865			1.00		3.1	
	50	ATOM	32		LEU	310		4.993	14.905			1.00		3.1	
	30	ATOM	33	N	THR	311		3.352	13.64			1.00		L.7	
		ATOM	34	CA	THR	311		3.017	14.604			1.00		9.9	
		ATOM	35	CB	THR	311		1.527	14.554			1.00		3.9	
		ATOM	36	OG1	THR	311		1.242	13.31			1.00		7.2	
	55	ATOM	37	CG2	THR	311		0.666	14.688			1.00).9	
	33	ATOM	38	C	THR	311		3.815	14.201			1.00		3.8	
		ATOM	39	0	THR	311		4.371	13.103			1.00		5.6	
		ATOM	40	N G3	ALA	312		3.857	15.078			1.00		3.7	
		ATOM ATOM	41 42	CA CB	ALA	312		4.590	14.798			1.00		7.7	
	60	ATOM	42 43	CB	ALA ALA	312 312		4.359 4.171	15.910 13.460			1.00		7.0	
	00			0					12.609					7.4	
		MOTA	44	U	ALA	312		5.009	12.005	18.26	4	1.00	4:	5.5	4

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	5	ATOM	45	N	ASP	313	2.868	13.275	18.143	1.00	47.58
		ATOM	46	CA	ASP	313	2.367	12.032	18.714	1.00	47.63
		ATOM	47	CB	ASP	313	0.848	12.100	18.879		. 51.96
		MOTA	48	CG	ASP	313	0.430	12.872	20.118	1.00	56.21
		ATOM	49	OD1	ASP	313	1.314	13.234	20.118	1.00	56.38
	10										
	10	ATOM	50	OD2	ASP	313	-0.785	13.117	20.282	1.00	59.15
		ATOM	51	C	ASP	313	2.745	10.846	17.835	1.00	43.93
		ATOM	52	0	ASP	313	2.959	9.741	18.330	1.00	44.77
		ATOM	53	N	GLN	314	2.826	11.081	16.531	1.00	44.52
		ATOM	54	CA	GLN	314	3.182	10.028	15.588	1.00	44.73
	15	ATOM	55	CB	GLN	314	2.849	10.464	14.156	1.00	45.05
		ATOM	56	CG	GLN	314	1.534	9.886	13.626	1.00	48.47
		ATOM	57	CD	GLN	314	0.982	10.646	12.428	1.00	50.37
		ATOM	58	OE1	GLN	314	1.649	11.515	11.856	1.00	49.38
		ATOM	59	NE2	GLN	314	-0.248	10.318	12.043	1.00	51.74
	20	ATOM	60	C	GLN						
	20					314	4.673	9.722	15.707	1.00	43.26
		ATOM	61	0	GLN	314	5.100	8.580	15.555	1.00	43.93
		ATOM	62	N	MET	315	5.459	10.757	15.980	1.00	42.29
		ATOM	63	CA	MET	315	6.901	10.606	16.130	1.00	41.26
		MOTA	64	CB	MET	315	7.565	11.985	16.224	1.00	42.43
- ats	25	ATOM	65	CG	MET	315	9.082	11.939	16.356	1.00	42.34
		MOTA	66	SD	MET	315	9.906	11.190	14.925	1.00	46.22
		ATOM	67	CE	MET	315	9.547	12.408	13.680	1.00	37.32
14		ATOM	68	С	MET	315	7.218	9.791	17.379	1.00	38.89
I		ATOM	69	0	MET	315	8.002	8.841	17.335	1.00	40.02
	30	ATOM	70	N	VAL	316	6.599	10.165	18.491	1.00	37.65
, , , , ,	20	ATOM	71	CA	VAL	316	6.819	9.476	19.756	1.00	39.56
		ATOM	72	CB	VAL	316	6.023	10.136	20.897	1.00	39.22
4.		ATOM	73	CG1	VAL	316	6.245	9.373	22.192	1.00	44.43
3 :		ATOM	74	CG2	VAL	316		11.583			
	35						6.446		21.059	1.00	41.04
	33	ATOM	75 76	C	VAL	316	6.404	8.012	19.664	1.00	40.04
1.1		MOTA	76	0	VAL	316	7.141	7.117	20.077	1.00	37.86
		MOTA	77	N	SER	317	5.215	7.767	19.127	1.00	41.90
ind de		MOTA	78	CA	SER	317	4.733	6.400	18.997	1.00	41.68
I		MOTA	79	CB	SER	317	3.311	6.402	18.415	1.00	43.85
£	40	ATOM	80	OG	SER	317	3.225	5.631	17.230	1.00	49.38
		MOTA	81	C	SER	317	5.696	5.601	18.114	1.00	39.72
		ATOM	82	0	SER	317	6.011	4.446	18.407	1.00	40.21
		ATOM	83	N	ALA	318	6.182	6.220	17.043	1.00	38.35
		ATOM	84	CA	ALA	318	7.114	5.540	16.153	1.00	36.96
	45	ATOM	85	CB	ALA	318	7.485	6.448	14.986	1.00	37.92
		ATOM	86	С	ALA	318	8.375	5.137	16.920	1.00	38.31
		ATOM	87	0	ALA	318	8.820	3.992	16.844	1.00	33.94
		ATOM	88	N	LEU	319	8.938	6.089	17.664	1.00	36.92
	50	ATOM	89	CA	LEU	319	10.161	5.854	18.438	1.00	38.56
	50	ATOM	90	CB	LEU	319	10.660	7.174	19.040	1.00	40.86
		MOTA	91	CG	LEU	319	11.136	8.264	18.071	1.00	41.25
		ATOM	92	CD1	LEU	319	11.714	9.440	18.857	1.00	44.30
		MOTA	93	CD2	LEU	319	12.182	7.693	17.140	1.00	42.61
		MOTA	94	C	LEU	319	9.965	4.826	19.549	1.00	38.33
	55	MOTA	95	0	LEU	319	10.779	3.916	19.729	1.00	33.91
		MOTA	96	N	LEU	320	8.879	4.982	20.297	1.00	37.39
		MOTA	97	CA	LEU	320	8.567	4.067	21.387	1.00	41.55
		ATOM	98	CB	LEU	320	7.239	4.467	22.049	1.00	38.47
		ATOM	99	CG	LEU	320	7.236	5.582	23.099	1.00	44.81
	60	ATOM	100	CD1	LEU	320	5.876	5.634	23.802	1.00	44.96
		ATOM	101	CD2	LEU	320	8.334	5.332	24.112	1.00	43.36
			102	C		320					
		MOTA	102	C	LEU	<i>3</i> ∠0	8.466	2.642	20.843	1.00	41.11

	_										
	5	ATOM	103	0	LEU	320	8.971	1.697	21.443	1.00	41.87
		ATOM	104	N	ASP	321	7.812	2.504	19.696	1.00	43.94
		ATOM	105	CA	ASP	321	7.613	1.210	19.053	1.00	44.77
		MOTA	106	CB	ASP	321	6.669	1.372	17.860	1.00	48.39
		ATOM	107	CG	ASP	321	5.206	1.318	18.255	1.00	52.39
	10	ATOM	108	OD1	ASP	321	4.901	1.422	19.464	1.00	53.56
		ATOM	109	OD2	ASP	321	4.357	1.172	17.346	1.00	55.81
		ATOM	110	C	ASP	321		0.565			
							8.911		18.568	1.00	44.37
		ATOM	111	0	ASP	321	9.030	-0.661	18.533	1.00	44.67
		ATOM	112	N	ALA	322	9.878	1.395	18.193	1.00	40.75
	15	ATOM	113	CA	ALA	322	11.153	0.905	17.686	1.00	37.81
		ATOM	114	CB	ALA	322	11.772	1.954	16.776	1.00	38.07
		ATOM	115	C	ALA	322	12.148	0.513	18.769	1.00	35.52
		MOTA	116	0	ALA	322	13.219	-0.020	18.473	1.00	36.11
		MOTA	117	N	${ t GLU}$	323	11.799	0.768	20.022	1.00	35.61
	20	MOTA	118	CA	${ t GLU}$	323	12.704	0.460	21.117	1.00	36.39
		ATOM	119	CB	GLU	323	12.042	0.768	22.459	1.00	35.09
		ATOM	120	CG	GLU	323	12.209	2.210	22.899	1.00	37.93
		ATOM	121	CD	GLU	323	13.657	2.569	23.200	1.00	37.29
		ATOM	122	OE1	GLU	323	14.313	3.173	22.326	1.00	34.21
4 2002	25	MOTA	123	OE2	GLU	323	14.134	2.245	24.309	1.00	38.02
1_4		MOTA	124	C	GLU	323	13.205	-0.978	21.110	1.00	38.01
		MOTA	125	0	GLU	323	12.425	-1.931	20.999	1.00	38.37
ij.		ATOM	126	N	PRO	324	14.527	-1.151	21.225	1.00	36.03
	20	MOTA	127	CD	PRO	324	15.522	-0.069	21.345	1.00	36.69
	30	MOTA	128	CA	PRO	324	15.158	-2.474	21.240	1.00	36.42
**************************************		ATOM	129	CB	PRO	324	16.633	-2.166	21.003	1.00	35.75
		ATOM	130	CG	PRO	324	16.811	-0.807	21.610	1.00	35.46
÷		ATOM	131	С	PRO	324	14.940	-3.162	22.583	1.00	35.75
5:		ATOM	132	Ō		324					
	25				PRO		14.616	-2.517	23.580	1.00	34.97
E . E	35	ATOM	133	N	PRO	325	15.134	-4.485	22.631	1.00	35.24
9.4		MOTA	134	CD	PRO	325	15.530	-5.386	21.534	1.00	37.02
144		ATOM	135	CA	PRO	325	14.942	-5.208	23.889	1.00	34.65
		ATOM	136	CB	PRO	325	14.753	-6.652	23.439	1.00	35.83
		ATOM	137	CG	PRO	325	15.589	-6.743	22.200	1.00	34.88
	40	ATOM	138	C	PRO	325	16.132	-5.070	24.824	1.00	34.51
	10										
		ATOM	139	0	PRO	325	17.237	-4.723	24.399	1.00	29.92
		ATOM	140	N	ILE	326	15.899	-5.322	26.106	1.00	33.62
		MOTA	141	CA	ILE	326	16.975	-5.265	27.075	1.00	35.02
		ATOM	142	CB	ILE	326	16.458	-4.891	28.473	1.00	38.11
	45	MOTA	143	CG2	ILE	326	17.557	-5.110	29.504	1.00	38.70
		ATOM	144	CG1	ILE	326	15.987	-3.431	28.466	1.00	40.48
		ATOM	145	CD1	ILE	326	16.035	-2.747	29.815	1.00	42.96
		MOTA	146	С	ILE	326	17.567	-6.668	27.103	1.00	34.14
		ATOM	147	0	ILE	326	16.875	-7.634	27.427	1.00	34.88
	50	MOTA	148	N	LEU	327	18.840	-6.784	26.745	1.00	29.64
		MOTA	149	CA	LEU	327	19.493	-8.083	26.716	1.00	29.54
		MOTA	150	CB	LEU	327	20.528	-8.135	25.587	1.00	27.76
		MOTA	151	CG	LEU	327	19.978	-7.800	24.196	1.00	29.02
		MOTA	152	CD1	LEU	327	21.068	-7.993	23.139	1.00	28.76
	55	MOTA	153	CD2	LEU	327	18.775	-8.688	23.891	1.00	31.26
		MOTA	154	C	LEU	327	20.156	-8.438	28.030	1.00	31.21
		ATOM	155	0	LEU	327	20.393	-7.578	28.891	1.00	30.12
		ATOM	156	N	TYR	328	20.445	-9.725	28.181	1.00	30.99
	C C	MOTA	157	CA	TYR	328		-10.229	29.381	1.00	30.95
	60	MOTA	158	CB	TYR	328		-11.520	29.842	1.00	33.38
		ATOM	159	CG	TYR	328	19.194	-11.272	30.686	1.00	33.05
		ATOM	160	CD1	TYR	328	19.253	-11.398	32.071	1.00	31.92
							23.230				

	5	ATOM	1031	0	PHE	435	16.361	12.135	35.496	1.00	34.78
		MOTA	1032	N	ARG	436	17.968	11.216	36.777	1.00	38.27
		ATOM	1033	CA	ARG	436	17.094	10.982	37.924	1.00	40.67
		ATOM	1034	CB	ARG	436	17.844	10.215	39.012	1.00	40.70
		ATOM	1035	CG	ARG	436	16.942	9.590	40.068	1.00	44.98
	10	ATOM	1036	CD	ARG	436	17.648	8.459	40.810	1.00	48.09
		ATOM	1037	NE	ARG	436	18.982	8.841	41.275	1.00	50.16
		ATOM	1038	CZ	ARG	436	20.119	8.361	40.777	1.00	52.19
		ATOM	1039	NH1	ARG	436	20.099	7.472	39.790	1.00	49.34
		ATOM	1040	NH2	ARG	436	21.283	8.770	41.266	1.00	51.85
	15	ATOM	1041	С	ARG	436	16.576	12.302	38.493	1.00	40.40
		ATOM	1042	0	ARG	436	15.382	12.458	38.730	1.00	41.49
		ATOM	1043	N	MET	437	17.477	13.252	38.706	1.00	40.02
		MOTA	1044	CA	MET	437	17.090	14.546	39.245	1.00	41.02
	•	ATOM	1045	CB	MET	437	18.329	15.427	39.440	1.00	40.29
	20	MOTA	1046	C	MET	437	16.099	15.221	38.299	1.00	40.81
		MOTA	1047	0	MET	437	15.111	15.805	38.734	1.00	42.46
		MOTA	1048	N	MET	438	16.367	15.127	37.001	1.00	39.02
		ATOM	1049	CA	MET	438	15.510	15.732	35.988	1.00	40.11
	25	ATOM	1050	CB	MET	438	16.237	15.793	34.651	1.00	38.16
	25	ATOM	1051	CG	MET	438	17.352	16.794	34.601	1.00	41.52
ıİ		ATOM	1052	SD	MET	438	17.999	16.862	32.943	1.00	43.94
of the control of the		ATOM	1053	CE	MET	438	16.698	17.748	32.096	1.00	39.96
IJ		ATOM	1054	C	MET	438	14.221	14.964	35.783	1.00	37.72
	30	MOTA	1055	0	MET	438	13.305	15.451	35.125	1.00	36.82
	30	ATOM	1056	N	ASN	439	14.155	13.759	36.337	1.00	38.81
į i		ATOM ATOM	1057 1058	CA CB	ASN ASN	439 439	12.981	12.919 13.556	36.174 36.847	1.00	40.77 44.52
		ATOM	1050	СБ СG	ASN ASN	439	11.762 10.566	12.620	36.887	1.00	44.52
91		ATOM	1060	OD1	ASN	439	10.300	11.400	36.964	1.00	48.48
	35	ATOM	1061	ND2	ASN	439	9.365	13.189	36.829	1.00	50.23
IJ	33	ATOM	1062	C	ASN	439	12.725	12.744	34.677	1.00	39.36
		ATOM	1063	0	ASN	439	11.637	13.037	34.172	1.00	37.76
and and and and and and and and and and		ATOM	1064	N	LEU	440	13.749	12.274	33.972	1.00	37.65
ı,		ATOM	1065	CA	LEU	440	13.655	12.052	32.532	1.00	35.22
	40	ATOM	1066	СВ	LEU	440	14.999	11.576	31.987	1.00	34.70
		ATOM	1067	CG	LEU	440	15.022	11.467	30.462	1.00	35.45
		ATOM	1068	CD1	LEU	440	14.890	12.862	29.869	1.00	35.24
		MOTA	1069	CD2	LEU	440	16.297	10.795	29.999	1.00	35.30
		MOTA	1070	C	LEU	440	12.587	11.024	32.196	1.00	36.48
	45	MOTA	1071	0	LEU	440	12.518	9.967	32.826	1.00	37.36
		MOTA	1072	N	GLN	441	11.763	11.328	31.197	1.00	36.82
		MOTA	1073	CA	GLN	441	10.696	10.420	30.785	1.00	38.51
		MOTA	1074	CB	GLN	441	9.431	11.211	30.443	1.00	38.23
		MOTA	1075	CG	GLN	441	8.912	12.063	31.592	1.00	42.46
	50	MOTA	1076	CD	GLN	441	8.362	11.227	32.729	1.00	44.91
		MOTA	1077	OE1	GLN	441	7.268	10.668	32.629	1.00	47.31
		MOTA	1078	NE2	GLN	441	9.119	11.132	33.818	1.00	44.06
		ATOM	1079	C	GLN	441	11.099	9.565	29.585	1.00	38.48
		MOTA	1080	0	GLN	441	11.923	9.976	28.763	1.00	35.80
	55	MOTA	1081	N	GLY	442	10.500	8.378	29.494	1.00	36.03
		MOTA	1082	CA	GLY	442	10.792	7.468	28.401	1.00	37.72
		MOTA	1083	C	GLY	442	10.599	8.112	27.043	1.00	36.88
		ATOM	1084	0	GLY	442	11.381	7.877	26.123	1.00	33.72
	60	ATOM	1085	N	GLU	443	9.556	8.925	26.918	1.00	36.59
	UU	ATOM	1086	CA	GLU	443	9.269	9.603	25.661	1.00	37.13
		ATOM	1087 1088	CB CG	GLU GLU	443 443	7.956	10.379 9.488	25.764 25.879	1.00	41.57
		ATOM	TOOR	CG	GLU	443	6.723	7.488	23.8/9	1.00	47.76

	5	ATOM	1089	CD	GLU	443	6.483	9.008	27.302	1.00	53.96
		ATOM	1090	OE1	GLU	443	5.619	8.123	27.498	1.00	57.66
		ATOM	1091	OE2	GLU	443	7.159	9.515	28.225	1.00	56.13
		ATOM	1092	С	GLU	443	10.408	10.551	25.311	1.00	35.27
		ATOM	1093	0	GLU	443	10.759	10.704	24.145	1.00	33.85
	10	ATOM	1094	N	GLU	444	10.984	11.179	26.331	1.00	32.09
		ATOM	1095	CA	GLU	444	12.097	12.095	26.126	1.00	33.92
		ATOM	1096	CB	GLU	444	12.332	12.924	27.388	1.00	34.97
		MOTA	1097	CG	GLU	444	11.169	13.845	27.732	1.00	38.28
		ATOM	1098	CD	GLU	444	11.383	14.610	29.023	1.00	38.11
	15	ATOM	1099	OE1	GLU	444	11.800	13.993	30.026	1.00	39.53
		ATOM	1100	OE2	GLU	444	11.132	15.834	29.036	1.00	40.77
		ATOM	1101	С	GLU	444	13.356	11.305	25.770	1.00	33.59
		ATOM	1102	0	GLU	444	14.085	11.670	24.842	1.00	33.35
		ATOM	1103	N	PHE	445	13.590	10.215	26.501	1.00	30.68
	20	ATOM	1104	CA	PHE	445	14.753	9.357	26.276	1.00	32.49
		ATOM	1105	CB	PHE	445	14.703	8.139	27.203	1.00	29.35
		ATOM	1106	CG	PHE	445	15.667	7.047	26.828	1.00	30.78
		ATOM	1107	CD1	PHE	445	17.036	7.201	27.030	1.00	28.25
		MOTA	1108	CD2	PHE	445	15.205	5.863	26.266	1.00	30.62
	25	MOTA	1109	CE1	PHE	445	17.933	6.195	26.675	1.00	28.67
last Little		MOTA	1110	CE2	PHE	445	16.095	4.848	25.908	1.00	31.37
		MOTA	1111	CZ	PHE	445	17.460	5.015	26.113	1.00	30.37
		MOTA	1112	С	PHE	445	14.850	8.885	24.829	1.00	31.11
124 <i>8</i> 8_4		ATOM	1113	0	PHE	445	15.924	8.947	24.221	1.00	32.20
4	30	ATOM	1114	N	VAL	446	13.739	8.415	24.266	1.00	28.63
		MOTA	1115	CA	VAL	446	13.787	7.943	22.889	1.00	27.94
1		ATOM	1116	CB	VAL	446	12.478	7.193	22.478	1.00	28.48
		ATOM	1117	CG1	VAL	446	12.318	5.939	23.343	1.00	29.61
51 2004		ATOM	1118	CG2	VAL	446	11.265	8.092	22.607	1.00	27.23
	35	ATOM	1119	С	VAL	446	14.099	9.064	21.900	1.00	27.28
8 2 8		MOTA	1120	0	VAL	446	14.781	8.837	20.904	1.00	28.07
		MOTA	1121	N	CYS	447	13.619	10.275	22.166	1.00	28.97
i diagram		MOTA	1122	CA	CYS	447	13.919	11.394	21.272	1.00	29.14
i de		MOTA	1123	CB	CYS	447	13.156	12.653	21.693	1.00	28.90
" start	40	MOTA	1124	SG	CYS	447	11.389	12.591	21.309	1.00	35.68
		ATOM	1125	С	CYS	447	15.420	11.677	21.328	1.00	28.03
		MOTA	1126	0	CYS	447	16.063	11.885	20.302	1.00	29.34
		MOTA	1127	N	LEU	448	15.969	11.686	22.538	1.00	27.28
	4.5	MOTA	1128	CA	LEU	448	17.392	11.938	22.729	1.00	25.30
	45	MOTA	1129	CB	LEU	448	17.733	11.932	24.220	1.00	27.72
		ATOM	1130	CG	LEU	448	17.248	13.135	25.040	1.00	29.54
		ATOM	1131	CD1	LEU	448	17.807	13.042	26.454	1.00	30.85
		MOTA	1132	CD2	LEU	448	17.688	14.434	24.376	1.00	30.24
	50	MOTA	1133	C	LEU	448	18.245	10.902	22.008	1.00	27.62
	50	ATOM	1134	0	LEU	448	19.207	11.252	21.327	1.00	25.10
		ATOM	1135	N	LYS	449	17.905	9.621	22.162	1.00	25.16
		ATOM	1136	CA	LYS	449	18.673	8.570	21.506	1.00	27.55
		MOTA	1137	CB	LYS	449	18.135	7.185	21.900	1.00	28.99
	<i></i>	MOTA	1138	CG	LYS	449	19.134	6.052	21.694	1.00	34.70
	55	MOTA	1139	CD	LYS	449	18.737	4.789	22.459	1.00	32.67
		MOTA	1140	CE	LYS	449	17.267	4.419	22.220	1.00	31.87
		MOTA	1141	NZ	LYS	449	17.022	2.967	22.472	1.00	29.14
		MOTA	1142	C	LYS	449	18.626	8.749	19.990	1.00	25.88
	60	MOTA	1143	O	LYS	449	19.610	8.489	19.296	1.00	25.93
	UU	ATOM	1144	N C7	SER	450 450	17.482	9.197	19.480	1.00	26.07
		ATOM	1145	CA CB	SER SER	450 450	17.323 15.857	9.421 9.705	18.052 17.721	1.00 1.00	27.24 32.24
		ATOM	1146	CD	9rk	430	13.03/	J. 1UO	1/./21	1.00	34.44

	5	MOTA	1379	N	ASP	480	15.076	18.703	13.415	1.00	31.52
		MOTA	1380	CA	ASP	480	14.162	19.800	13.741	1.00	33.84
		MOTA	1381	CB	ASP	480	13.943	20.712	12.528	1.00	34.37
		MOTA	1382	CG	ASP	480	15.055	21.743	12.345	1.00	36.26
		ATOM	1383	OD1	ASP	480	15.119	22.354	11.257	1.00	36.56
	10	MOTA	1384	OD2	ASP	480	15.860	21.951	13.274	1.00	34.19
		ATOM	1385	С	ASP	480	12.818	19.222	14.174	1.00	33.48
		ATOM	1386	0	ASP	480	12.186	19.724	15.105	1.00	33.89
		ATOM	1387	N	LYS	481	12.379	18.161	13.498	1.00	33.90
		MOTA	1388	CA	LYS	481	11.106	17.536	13.839	1.00	32.97
	15	ATOM	1389	CB	LYS	481	10.719	16.489	12.784	1.00	34.66
		ATOM	1390	С	LYS	481	11.164	16.895	15.225	1.00	33.57
		ATOM	1391	0	LYS	481	10.167	16.869	15.943	1.00	35.37
		ATOM	1392	N	ILE	482	12.328	16.377	15.607	1.00	32.71
		ATOM	1393	CA	ILE	482	12.457	15.764	16.922	1.00	31.60
	20	ATOM	1394	CB	ILE	482	13.743	14.913	17.028	1.00	32.65
		ATOM	1395	CG2	ILE	482	13.877	14.338	18.430	1.00	32.50
		ATOM	1396	CG1	ILE	482	13.697	13.785	15.995	1.00	32.72
		ATOM	1397	CD1	ILE	482	14.978	12.969	15.908	1.00	33.37
		ATOM	1398	С	ILE	482	12.456	16.853	17.994	1.00	31.69
	25	ATOM	1399	0	ILE	482	11.946	16.649	19.097	1.00	29.98
and des		ATOM	1400	N	THR	483	13.027	18.012	17.679	1.00	31.33
		ATOM	1401	CA	THR	483	13.022	19.109	18.644	1.00	31.71
		ATOM	1402	СВ	THR	483	13.756	20.351	18.109	1.00	32.92
AJ så		ATOM	1403	OG1	THR	483	15.111	20.012	17.788	1.00	29.99
	30	ATOM	1404	CG2	THR	483	13.756	21.452	19.160	1.00	30.47
		ATOM	1405	С	THR	483	11.559	19.483	18.920	1.00	32.85
		ATOM	1406	0	THR	483	11.146	19.598	20.070	1.00	31.83
1,71		ATOM	1407	N	ASP	484	10.785	19.656	17.851	1.00	31.91
: ::t::::		ATOM	1408	CA	ASP	484	9.369	20.003	17.965	1.00	34.15
ud :	35	ATOM	1409	СВ	ASP	484	8.708	20.013	16.591	1.00	37.41
IJ.		ATOM	1410	CG	ASP	484	9.270	21.080	15.680	1.00	42.02
IJ		ATOM	1411	OD1	ASP	484	9.871	22.045	16.198	1.00	43.26
أعد عد		ATOM	1412	OD2	ASP	484	9.106	20.952	14.445	1.00	42.49
		ATOM	1413	C	ASP	484	8.657	18.985	18.840	1.00	33.16
ij	40	ATOM	1414	0	ASP	484	7.830	19.339	19.676	1.00	34.86
		MOTA	1415	N	THR	485	8.996	17.715	18.646	1.00	33.91
		MOTA	1416	CA	THR	485	8.396	16.635	19.414	1.00	34.41
		MOTA	1417	CB	THR	485	8.875	15.268	18.885	1.00	33.58
		ATOM	1418	OG1	THR	485	8.400	15.094	17.542	1.00	37.04
	45	ATOM	1419	CG2	THR	485	8.347	14.138	19.751	1.00	30.89
		ATOM	1420	С	THR	485	8.708	16.757	20.903	1.00	35.15
		ATOM	1421	0	THR	485	7.818	16.600	21.744	1.00	31.99
		MOTA	1422	N	LEU	486	9.966	17.046	21.229	1.00	33.77
		ATOM	1423	CA	LEU	486	10.368	17.192	22.621	1.00	34.31
	50	ATOM	1424	CB	LEU	486	11.879	17.448	22.721	1.00	32.00
		ATOM	1425	CG	LEU	486	12.776	16.201	22.754	1.00	34.99
		ATOM	1426	CD1	LEU	486	14.233	16.613	22.521	1.00	32.65
		MOTA	1427	CD2	LEU	486	12.635	15.481	24.105	1.00	29.90
		MOTA	1428	С	LEU	486	9.597	18.348	23.256	1.00	34.87
	55	MOTA	1429	0	LEU	486	9.078	18.225	24.362	1.00	35.85
		ATOM	1430	N	ILE	487	9.513	19.469	22.548	1.00	35.59
		MOTA	1431	CA	ILE	487	8.787	20.625	23.064	1.00	36.79
		ATOM	1432	CB	ILE	487	8.890	21.826	22.095	1.00	37.32
		ATOM	1433	CG2	ILE	487	7.833	22.884	22.443	1.00	40.19
	60	ATOM	1434		ILE	487	10.292	22.443	22.181	1.00	36.00
		ATOM	1435	CD1	ILE	487	10.635	23.041	23.544		33.58
		ATOM	1436	С	ILE	487	7.315	20.257	23.276	1.00	38.56

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METHODS AND COMPOUNDS FOR MODULATING NUCLEAR RECEPTOR COACTIVATOR BINDING

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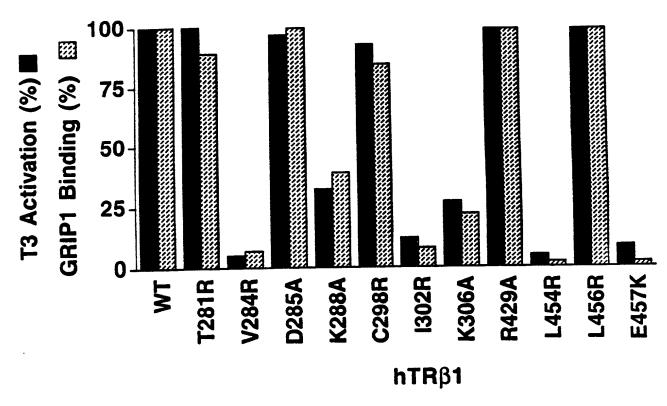
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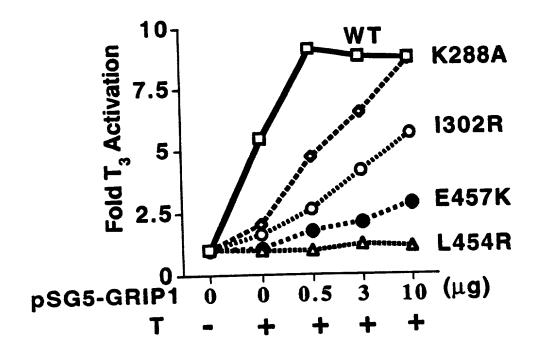
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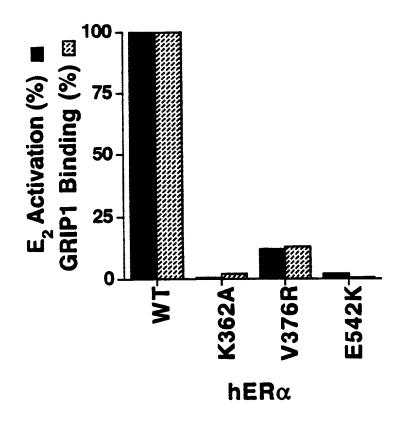
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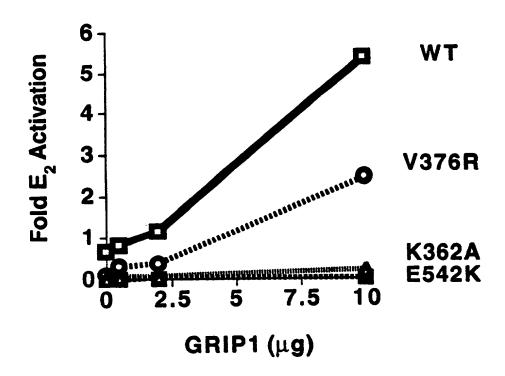
ABSTRACT OF THE DISCLOSURE

The present invention relates to methods and agonist/antagonist compounds for modulating nuclear receptor coactivator binding. The invention includes a method for identifying residues comprising a coactivator binding site for a nuclear receptor of interest. Also included is a method of identifying agonists and/or antagonists that bind to a coactivator binding site of a nuclear receptor of interest. Agonists and antagonists of coactivator binding to nuclear receptors also are provided. The invention is exemplified by identification and manipulation of the coactivator binding site of the thyroid receptor (TR), and compounds that bind to this sites. The methods can be applied to other nuclear receptors including RAR, RXR, PPAR, VDR, ER, GR, PR, MR, and AR.









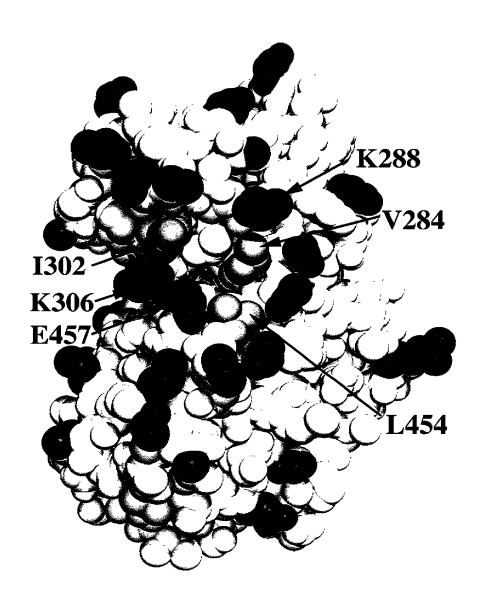


FIG. 5

Nuclear

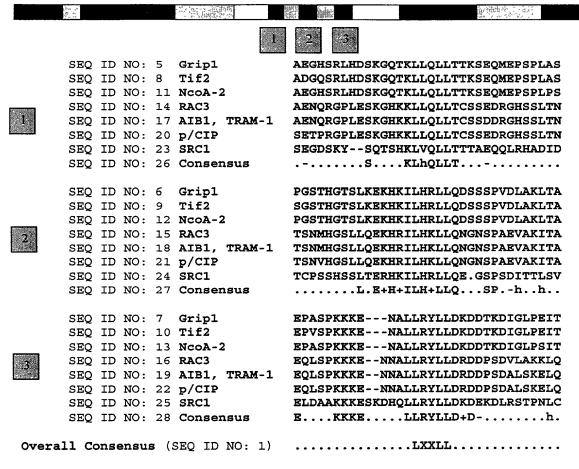
Receptor interaction

CBP & p300 interaction

bHLH PAS Transcriptional

activation

domain

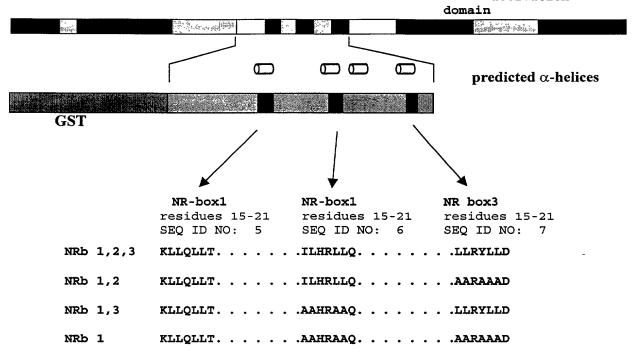


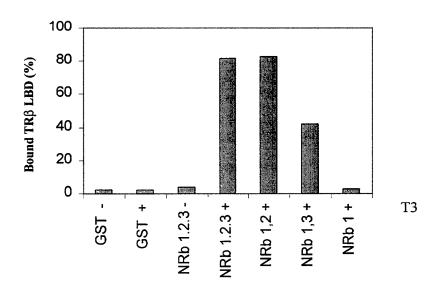
Nuclear Receptor interaction

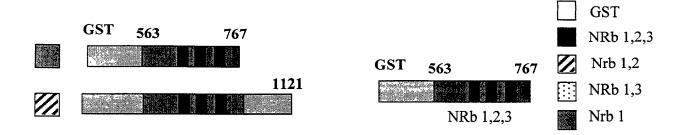
CBP & p300 interaction

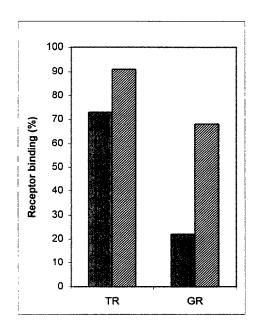
bHLH PAS Transcriptional

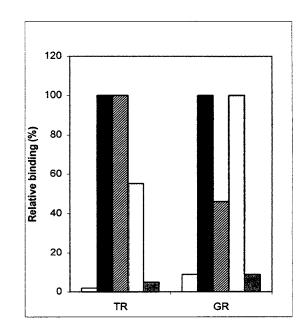
activation

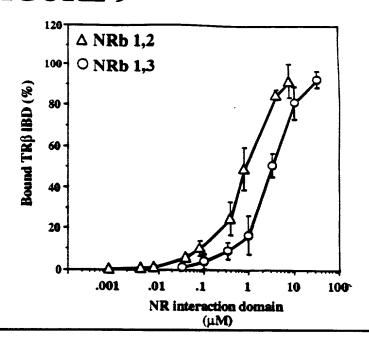


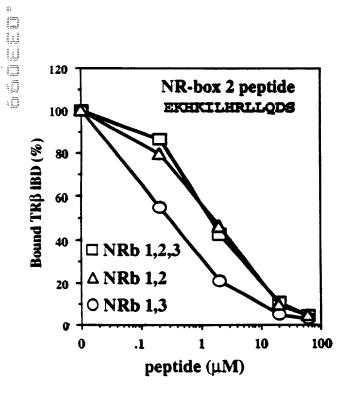


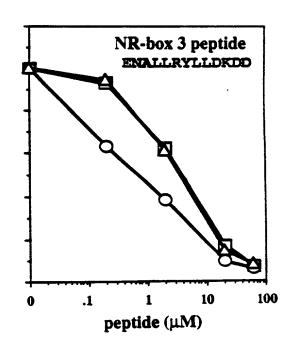


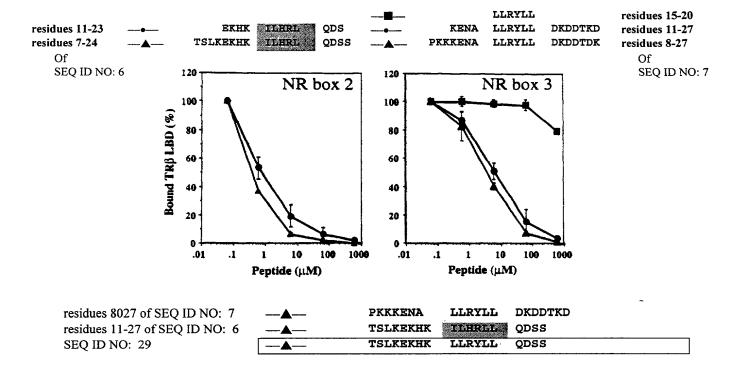


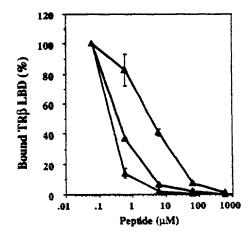


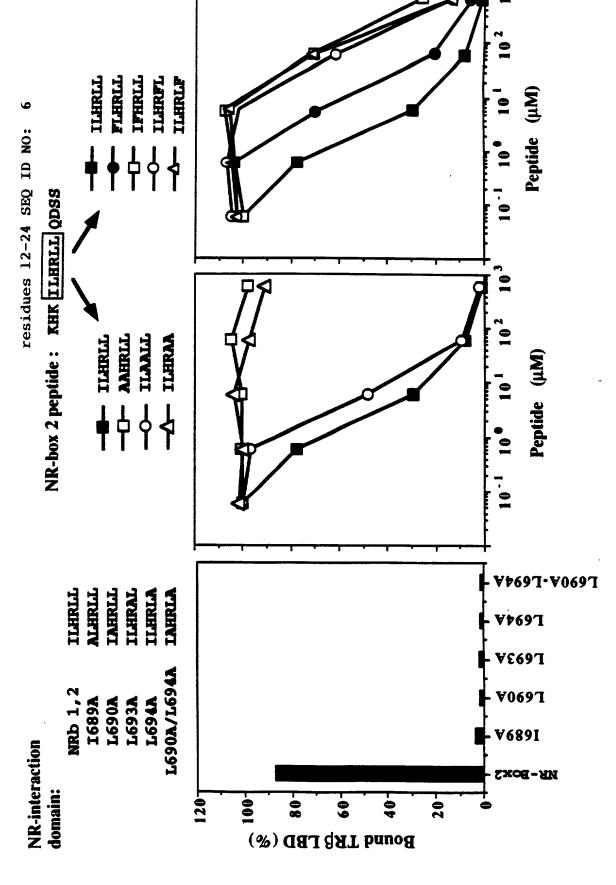












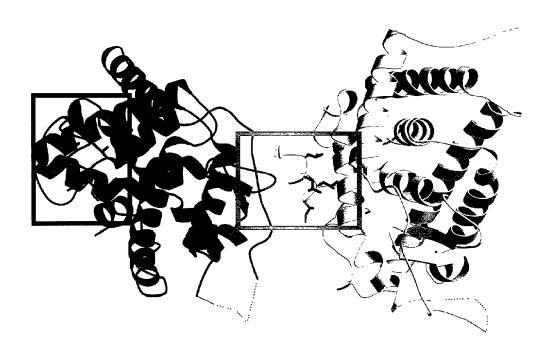


FIG. 12

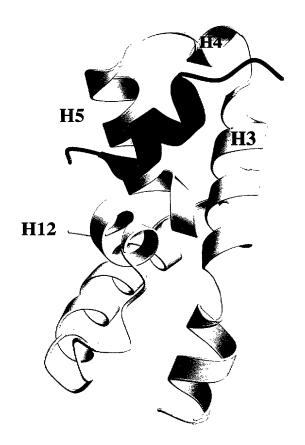


FIG. 13

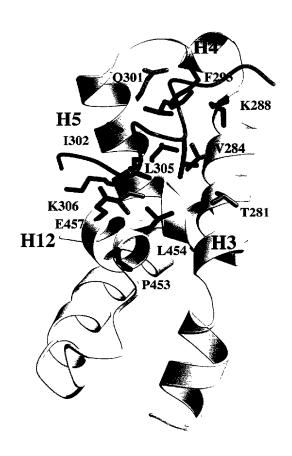


FIG. 14

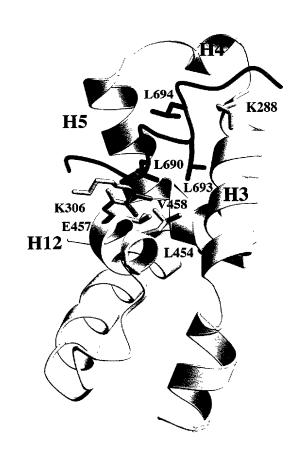


FIG. 15

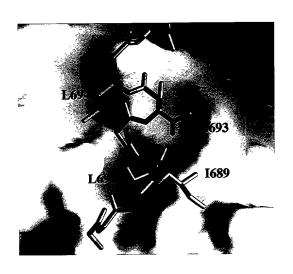


FIG. 16

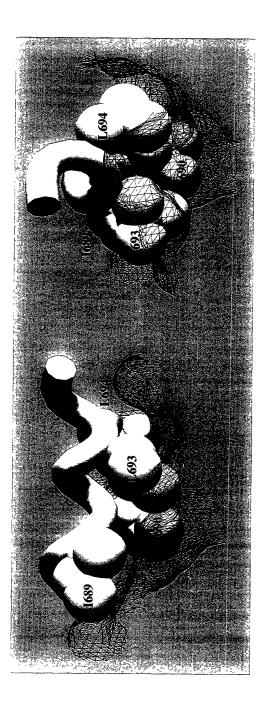


FIG. 17

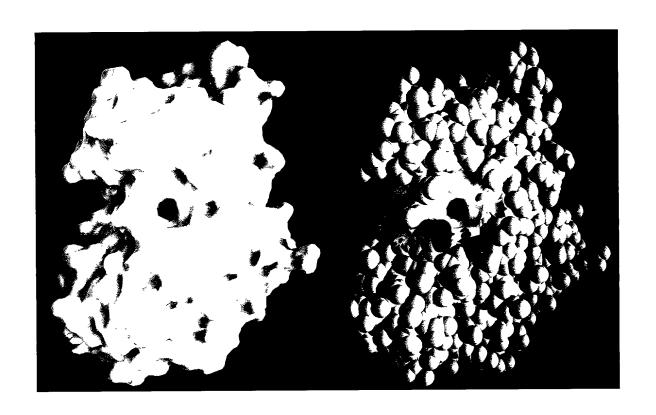


FIG. 18

FIGURE 19